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9. The methods of statistical analysis should be described in detail sufficient that a knowledgeable reader could reproduce the analysis if the data were available.

**From* Methodologic Guidelines for Reports of Clinical Trials (Cancer Treat Rep 69:1-3, 1985).

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555 ANNOUNCEMENTS

Evaluation of Antitumor Activity of 1-β-D-Arabinofuranosyl-2-amino-1,4(2*H*)-4-iminopyrimidine in Murine Tumors[1,2]

Shinobu Furusawa and Abdul Mohsin Mian[3,4,*]

1-β-D-Arabinofuranosyl-2-amino-1,4(2*H*)-4-iminopyrimidine (ara-AIPy), a new deaminase-resistant analog of cytarabine, exhibited extremely potent antitumor activity against P388 leukemia [400 mg/kg on Days 1–5; increase in life span (ILS), 211%] and significant inhibition against Lewis lung carcinoma (inhibition of tumor weight, 68%) and mammary adenocarcinoma 755 (inhibition of tumor weight, 82%). Schedule-dependency studies indicate that this drug, unlike cytarabine, was effective irrespective of its treatment schedules. The drug exhibited therapeutic efficacy against established P388 tumor transplants (400 mg/kg on Days 3–7; ILS, 131%) and inhibited the tumor growth effectively even when administered as a single dose on Day 1 by both ip (2000 mg/kg; ILS, 150%) and iv (500 mg/kg; ILS, 68%) routes. Ara-AIPy was most effective when administered on Days 1, 3, 5, 7, and 9 after tumor transplantation (400 mg/kg; ILS, 210%, with 50% of animals 60-day survivors). Ara-AIPy inhibited the growth of L1210 leukemia when both the tumor transplantation and the drug treatment were administered by iv route (500 mg/kg on Days 1, 5, and 9; ILS, 181%). The routes of administration of ara-AIPy experiments showed that the drug was effective by both ip and iv routes of administration; however, better therapeutic values were obtained by ip schedules. These studies demonstrate that ara-AIPy exhibits highly significant and broad-spectrum antitumor activity against a variety of experimental animal tumor models and suggest a possible future role for this drug in the treatment of human neoplasia. [Cancer Treat Rep 71:441–446, 1987]

Cytarabine (ara-C) has been used clinically for the treatment of acute leukemia and lymphomas (1–3). The antineoplastic activity of ara-C is attributed to the metabolite 1-β-D-arabinofuranosylcytosine 5′-triphosphate (ara-CTP) (4), which is a potent inhibitor of DNA polymerase (5,6), as well as to its incorporation into DNA (6–9). However, ara-C is rapidly metabolized by deoxycytidine deaminase to biologically inactive uracil arabinoside (10,11). Thus, a relatively short drug plasma half-life necessitates complex scheduling including continuous iv infusion. Attempts to produce deaminase-resistant derivatives of ara-C have resulted in a number of promising leads with pronounced antitumor activities in experimental animal models (12–15), but so far no clinically useful drug has emerged. 2′-Azido-2′-deoxyarabinofuranosylcytosine, a deaminase-resistant experimental drug, has been reported (16) to be curative against L1210 leukemia. However, a fully comprehensive evaluation of this derivative is still awaited. 2,2′-Anhydro-1-β-D-arabinofuranosylcytosine (anhydro-ara-C), another deaminase-resistant depot form of ara-C (13), has been shown to be highly effective in inhibiting the growth of a variety of transplantable rodent tumors (12,13). However, in clinical trials, anhydro-ara-C produced vascular instability and parotid pain which have limited the clinical use of this drug (17,18).

We earlier reported the synthesis and antileukemic activity of 1-β-D-arabinofuranosyl-2-amino-1,4(2*H*)-4-iminopyrimidine (ara-AIPy; fig 1) (19,20). Ara-AIPy represents a class of compounds which structurally resemble both ara-C and anhydro-ara-C, since it has a freely rotating arabinose sugar like ara-C and a quinonoide aglycone moiety like anhydro-ara-C. Ara-AIPy, a deaminase-resistant ara-C analog, has pronounced activity against L1210 leukemia (19,20). In comparative studies, ara-AIPy is greater than or equal to anhydro-ara-C

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[3] Papanicolaou Comprehensive Cancer Center and the Department of Oncology, University of Miami School of Medicine, FL.

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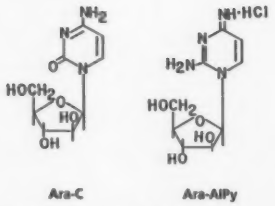

FIGURE 1.—Structures of ara-C and ara-AIPy.

and ara-C in inhibiting the growth of L1210 leukemia cells in vivo. These observations prompted us to further explore the potential of this drug as a new antitumor agent. In this paper, we report the tumor growth-inhibitory characteristics of this drug against a panel of experimental tumor models as well as the effects of routes of administration and treatment schedules on the therapeutic potency of ara-AIPy in murine leukemias.

MATERIALS AND METHODS

Drugs.—Ara-C was purchased from Sigma Chemical Co (St. Louis, MO). Anhydro-ara-C was prepared from cytidine by the method of Kikugawa and Ichino (21). Ara-AIPy was prepared from anhydro-ara-C according to our published methodology (19,20). Drugs were dissolved in phosphate-buffered saline (PBS) and were filtered through a 0.2-μm Millipore filter to obtain the sterile solutions. Final determinations of drug purity and exact concentration were made by spectrophotometric and high-performance liquid chromatographic analyses.

Animals.—C57BL/6, BDF_1, and DBA/2 female mice (6–8 weeks old; 20–22 g) used in these experiments were obtained from either The Jackson Laboratory (Bar Harbor, ME) or The Charles River Laboratory (Wilmington, MA). Animals were provided with Purina Laboratory feed and water ad libitum and were housed in a clean, air-conditioned environment.

Tumors.—P388 and L1210 leukemia and Lewis lung carcinoma cells were obtained from ATCC, Rockville, MD. Mammary adenocarcinoma 755 was supplied by Dr. S. Greer, Department of Microbiology, University of Miami. P388 and L1210 leukemia cells were maintained by weekly ip transplants of 10^6 and 10^5 cells, respectively, in DBA/2 female mice. Mammary adenocarcinoma 755 and Lewis lung carcinoma were maintained as serial sc tumor transplants (2–4-mm fragments in the left and right abdominal region) in C57BL/6 female mice. Tumor transplantations were carried out using standard protocols established by the Drug Evaluation Branch of the National Cancer Institute.

Evaluation of antitumor activity.—Tumor-bearing mice were treated with ara-AIPy, anhydro-ara-C, or ara-C by ip or iv route with inoculum of 0.01 ml/g of body weight at various drug concentrations and schedules of treatments as described in the tables for each set of experiments. Changes in body weight were used as an indicator of drug toxicity. In P388 and L1210 leukemias, the antitumor activity was established by the increase in life span (ILS) of drug-treated mice as compared with untreated controls using the formula

$$\text{ILS} = \frac{T - C}{C} \times 100,$$

where T = mean day of death of treated mice, and C = mean day of death of untreated control mice. In addition, the number of tumor cells surviving ara-AIPy treatment is also estimated. A direct relationship between the number of leukemic cells implanted (by various routes) and the life span in BDF_1 mice has been clearly demonstrated by Schabel et al (22). From their tabulated data, it is possible to estimate the number of leukemic cells surviving drug therapy reliably once the mean life span of treatment mice is obtained. The antitumor activities in mammary adenocarcinoma 755 and Lewis lung carcinoma were determined from the inhibition of tumor weight (TWI) 12 days after transplantation. Tumor weight (mg) was estimated by measuring length (l) and width (w) of each tumor with a vernier caliper (mm) and using the formula

$$\frac{l \times (w)^2}{2}$$

In vitro cytotoxicity studies.—P388 cells were grown as a suspension culture in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum and antibiotics at 37°C in 5% CO_2 atmosphere. The cytotoxic effects of ara-AIPy and ara-C were evaluated by treating 180 μl of P388 cells (10^5/ml) in triplicate with 20 μl of serially diluted drug solutions to produce 10^{-9} to 10^{-6} M final drug concentration in a 96-well flat-bottomed microtest plate (Nunclon #167,008; A/C Nunc, Roskilde, Denmark). After 48 hours, cell counts were determined with a Model ZB1 Coulter Counter and cell viability was determined by trypan blue dye exclusion. Inhibition of cell growth was determined by comparison with control cultures.

RESULTS

In Vitro Cytotoxicity Studies

Figure 2 shows that both ara-AIPy and ara-C caused dose-dependent growth inhibition at $> 10^{-9}$ M drug concentrations. The shapes of the dose-response curves for the two agents were similar but ara-AIPy was slightly less cytotoxic. The ID50 value of 6×10^{-8} M for ara-AIPy was higher than the ID50 of 3×10^{-8} M for ara-C.

In Vivo Studies Against P388 Leukemia

The antitumor activity of one daily administration (Days 1–5 ip) of different doses of ara-AIPy starting at 24 hours after ip tumor implant (10^6 cells/mouse) is shown in table 1. Ara-AIPy at 100–400 mg/kg exhibited in vivo tumor growth inhibition in a dose-dependent manner. These results show that the administration (Days 1–5 ip) of ara-AIPy (100–400 mg/kg) produced

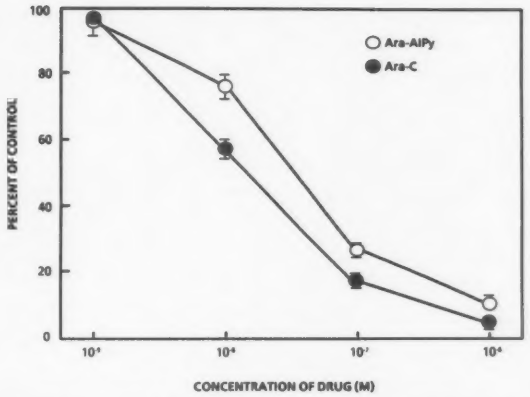

FIGURE 2.—Cytotoxic effects of ara-AIPy and ara-C against P388 leukemia cells. Cultures in triplicate were treated with ara-AIPy or ara-C for 48 hrs in drug concentrations shown. Results are expressed as % of untreated controls. Points = means of triplicate assays; bars = SD.

highly significant ILS values (72%–211%). The greatest ILS was observed at a dose of 400 mg/kg without any appreciable decrease in the host body weight. This treatment schedule produced six log cell kill and was almost curative. However, the next higher dose (800 mg/kg) exhibited host toxicity without any significant therapeutic value (data not shown). The use of anhydro-ara-C as a positive control in these experiments produced an ILS of 180% at 500-mg/kg dose regimens. These results demonstrate that the antileukemic activity of ara-AIPy is favorably comparable to that of anhydro-ara-C.

Schedule Dependency and Route of Administration Studies

To explore the optimal therapeutic effectiveness of ara-AIPy, schedule dependency and route of administration studies were carried out in ip transplanted P388 tumor-bearing animals. Data (table 2) show that the ip administration of 500, 1000, and 2000 mg/kg of ara-AIPy as single treatment on Day 1 resulted in ILS values of 79%, 110%, and 150%, respectively, whereas 4000 mg/kg was lethal. Similarly, 250 and 500 mg/kg of ara-AIPy when given via the tail vein as a single treatment on Day 1 produced ILS values of 32% and 68%, respectively. A single administration of 1000 mg/kg by iv route also resulted in death. Data for lethal toxic doses by both ip and iv administration are not shown, since both of these treatment schedules resulted in death for all of the animals within 24 hours of drug administration. These data clearly demonstrate the effectiveness of ara-AIPy, even when given in a single-dose treatment regimen. In fact, single injections of 1000 or 2000 mg/kg of ara-AIPy ip reduced tumor burden to less than five viable leukemic cells.

In another set of experiments, the efficacy of intermittent administration of this drug was evaluated. The results of these experiments indicate that ara-AIPy at 200- and 400-mg/kg drug concentrations, when given on Days 1, 3, 5, 7, and 9 ip, was an extremely effective schedule. The higher-dose treatment (400 mg/kg) resulted in cures for 50% of the animals (60-day survivals). To further determine the effectiveness of this agent against established P388 tumors, ara-AIPy treatment was initiated 72 hours after tumor transplant. In this schedule, a daily treatment of 200 and 400 mg/kg for 5 consecutive days started on Day 3 resulted in a significant ILS (108% and 131%, respectively) of tumor-bearing mice (table 2). These data (table 2) show that ara-AIPy is a highly effective antitumor agent irrespective of route of administration and treatment schedules.

Antitumor Activity Against L1210 Leukemia

The antileukemic activity of ara-AIPy administered ip against ip implanted L1210 has been established earlier (19,20). To determine the effectiveness of this drug where both the tumor transplantation and the drug administration were given by iv route, a set of experiments with two treatment schedules were carried out.

TABLE 1.—Effect of ara-AIPy on growth of P388 leukemia implanted in female BDF_1 mice*

Drug concentration (mg/kg ip)	Treatment days	Mean survival in days (± SD)	Mean No. of days after therapy	Δ wt on Day 5 (g)	% ILS	Estimated No. of tumor cells at end of therapy†
Control (PBS)	1–5	10.0 ± 0.7	—	+1.52	—	—
Ara-AIPy						
100	1–5	17.2 ± 1.7‡	12.2	+0.25	72	4.5×10^5
200	1–5	21.7 ± 2.2‡	16.7	+0.63	117	4.0×10^3
300	1–5	25.6 ± 3.4‡	20.6	−0.63	156	6.8×10^1
400	1–5	31.1 ± 3.5‡	26.1	−0.50	211	< 1
Anhydro-ara-C						
500	1–5	28.0 ± 4.2‡	23.0	−0.88	180	5×10^0

*P388 leukemia cells (1×10^6/mouse) implanted ip into female BDF_1 mice (8–10 mice/group) on Day 0; ara-AIPy or anhydro-ara-C administered ip daily for 5 days.

†Estimated from the data given by Schabel et al (22).

‡Significantly different from values for controls by Student's t test ($P < 0.01$).

TABLE 2.—Effect of various routes and schedules of administration of ara-AIPy on growth of P388 implanted in female BDF_1 mice*

Experiment	Drug concentration (mg/kg)	Treatment days	Mean survival in days (± SD)	Mean No. of days after therapy	Δ wt at end of treatment (g)†	% ILS	Estimated No. of tumor cells at end of therapy‡
A	Control (PBS)	1, 3, 5, 7, 9	10.1 ± 1.2	—	+3.50	—	—
	Ara-AIPy (ip)						
	200	1, 3, 5, 7, 9	24.3 ± 2.1§	15.3	+0.60	141	1.5×10^4
	400	1, 3, 5, 7, 9	31.3 ± 1.2§	22.3	+0.50	210¶	1.0×10^1
B	Control (PBS)	3–7	10.8 ± 1.4	—	+2.80	—	—
	Ara-AIPy (ip)						
	200	3–7	22.5 ± 2.3§	15.5	+0.20	108	1.3×10^4
	400	3–7	25.0 ± 1.4§	18.0	−0.40	131	9.8×10^2
C	Control (PBS)		11.5 ± 0.6	—	+0.22	—	—
	Ara-AIPy (ip)						
	500	1	20.6 ± 2.9§	19.6	+0.33	79	1.9×10^2
	1000	1	24.2 ± 4.8§	23.2	+0.13	110	4.0×10^0
	2000	1	28.8 ± 2.5§	27.8	−0.08	150	< 1
	Ara-AIPy (iv)						
	250	1	15.2 ± 2.1§	14.2	+0.47	32	5.2×10^4
	500	1	19.3 ± 2.3§	18.3	+0.48	68	9.0×10^2

*P388 leukemia cells (1×10^6/mouse) implanted ip into female BDF_1 mice (6–10 mice/group) on Day 0; ara-AIPy administered ip or iv by various schedules as shown.

†For Experiment A, average weight changes from Day 1 to Day 9 are indicated. For Experiment B, average weight changes from Day 3 to Day 7 are indicated. For Experiment C, average weight changes from Day 1 to Day 2 (24-hr period after drug administration) are indicated.

‡Estimated from the data given by Schabel et al (22).

§Significantly different from values for controls by Student's t test ($P < 0.01$).

¶3 of 6 mice were alive on Day 60; ILS calculations do not include survivors.

Ara-AIPy at a concentration of 250 and 500 mg/kg (table 3) when administered on Day 1 in a single-dose treatment significantly inhibited L1210 growth (ILS, 35% and 85%, respectively). Similarly, in a three-dose treatment schedule prescribed on Days 1, 5, and 9, ara-AIPy at 250 and 500 mg/kg produced significant ILS values of 110% and 181%, respectively.

Antitumor Activity Against Mammary Adenocarcinoma 755

Antitumor activity of ara-AIPy against sc implanted mammary adenocarcinoma 755 presented in table 4 shows that a daily treatment of ara-AIPy at 100-, 200-, and 400-mg/kg doses for 5 days (Days 1–5 ip) produced TWIs of 61%, 76%, and 82%, respectively. In the highest-dose (400 mg/kg) treatment regimen, one mouse did not have a palpable tumor. These results show that ara-AIPy has a highly significant growth-inhibitory effect on this tumor.

Antitumor Activity Against Lewis Lung Carcinoma

In table 5 data on the antitumor effect of ara-AIPy on Lewis lung carcinoma show that the five consecutive ip administrations of this drug at 100, 200, or 400 mg/kg

TABLE 3.—Effect of ara-AIPy on growth of L1210 leukemia implanted iv in female BDF_1 mice*

Drug concentration (mg/kg iv)	Treatment days	Mean survival in days (± SD)	Mean No. of days after therapy	Δ wt on Day 7 (g)	% ILS	Estimated No. of tumor cells at end of therapy†
Control (PBS)	1–5	8.4 ± 0.7	—	+0.45	—	—
Ara-AIPy						
250	1–5	11.3 ± 0.9‡	10.3	+0.08	35	5.2×10^2
500	1–5	15.5 ± 1.1‡	14.5	+0.10	85	< 1
Ara-AIPy						
250	1, 5, 9	17.6 ± 2.8‡	8.6	−0.25	110	1.1×10^4
500	1, 5, 9	23.6 ± 4.4‡	14.6	−0.17	181	< 1

*L1210 leukemia cells (1×10^4/mouse) implanted iv into tail vein of female BDF_1 mice (6–10 mice/group) on Day 0; ara-C or ara-AIPy administered iv on Day 1 or on Days 1, 5, and 9 after tumor transplant.

†Estimated from the data given by Schabel et al (22).

‡Significantly different from values for controls by Student's t test ($P < 0.01$).

TABLE 4.—Effect of ara-AIPy on growth of mammary adenocarcinoma 755 implanted (sc) in BDF_1 female mice*

Treatment (mg/kg ip)	Treatment days	Tumor wt on Day 12 ± SD (mg)	Δ wt on Day 5 (g)	Animals with palpable tumors on Day 12/total No.	% tumor growth inhibition
Control (PBS)	1–5	672 ± 154	+1.51	8/8	—
Ara-AIPy					
100	1–5	265 ± 65†	+0.62	6/6	61
200	1–5	164 ± 57†	+0.83	6/6	76
400	1–5	118 ± 34†	+0.07	5/6	82

*Groups of 6–8 female BDF_1 mice were inoculated sc with mammary adenocarcinoma 755 (2–4-mm fragments). Ara-AIPy was administered ip 24 hrs after transplantation (Days 1–5). Antitumor activity was determined from tumor weight on Day 12 as described in the Materials and Methods section.

†Significantly different from values for controls by Student's t test ($P < 0.01$).

starting on Day 1 produced significant inhibition of this tumor (TWIs of 53%, 59%, and 68%, respectively).

DISCUSSION

The facile susceptibility of ara-C to enzymatic deamination may have limited the full potential of its clinical usefulness as an antitumor agent. It has necessitated multiple injections or continuous infusion as a mode of its administration. To overcome the rapid catabolism and thus be able to administer the drug in a more desirable fashion, deaminase-resistant derivatives of ara-C with retention of its antitumor properties have been long sought after. However, very few such derivatives with clinical usefulness have emerged. Earlier, we reported the synthesis and antileukemic activities of a class of compounds with these desired features (19,20). In the present studies, we have shown that ara-AIPy inhibited the growth of P388 leukemia in vitro effectively (ID50, 6×10^{-8} M) but was somewhat less cytotoxic when compared to ara-C (ID50, 3×10^{-8} M). In animal model studies using P388 leukemia, ara-AIPy exhibited extremely potent antitumor activity (ILS, 211%; table 1) in a dose-dependent fashion, and the results are comparable to or better than those reported with anhydro-ara-C or ara-C (12,14). These results are in close agreement with the previously reported growth inhibitory effects of ara-AIPy against L1210 leukemia-bearing animals (20).

The antitumor activity of ara-C greatly depends on its schedule of administration, with optimal growth inhibitory effects being observed with frequent drug treatments against early tumors (23). However, our studies in the P388 tumor model show that ara-AIPy was effective irrespective of the treatment schedules and even against established tumors (table 2). The optimal therapeutic values were observed with intermittent treatment of ara-AIPy on Days 1, 3, 5, 7, and 9. In this treatment schedule (400 mg/kg), 50% of the animals were tumor-free on Day 60 (table 2). These results were greater than or equal to those reported (14) with anhydro-ara-C (ILS, 222%) or ara-C (ILS, 110%) despite the fact that both ara-C and anhydro-ara-C were administered for nine treatments (Days 1–9) as compared to five treatments of ara-AIPy. Similarly, a single treatment of ara-AIPy on Day 1 (optimal ILS, 150%) was significantly better than ara-C or anhydro-ara-C (reported optimal ILS, 44% and 56%, respectively) when given under similar schedules (14). In fact, single ip administration of ara-AIPy at 1000- or 2000-mg/kg con-

TABLE 5.—Effect of ara-AIPy on growth of Lewis lung carcinoma implanted sc in BDF_1 female mice*

Treatment (mg/kg ip)	Treatment Days	Tumor wt on Day 12 ± SD (mg)	Animals with palpable tumors on Day 12/total No.	% tumor growth inhibition
Control (PBS)	1–5	652 ± 136	10/10	—
Ara-AIPy				
100	1–5	307 ± 92†	6/6	53
200	1–5	268 ± 107†	6/6	59
400	1–5	210 ± 42†	6/6	68

*Groups of 6–10 female BDF_1 mice were inoculated sc with Lewis lung carcinoma (2–4-mm fragments). Ara-AIPy was administered ip 24 hours after transplantation (Days 1–5). Antitumor activity was calculated from tumor weight on Day 12 as described in the Materials and Methods section.

†Significantly different from values for controls by Student's t test ($P < 0.01$).

centrations appeared to reduce the animal tumor burden to fewer than five viable tumor cells. It is, therefore, anticipated that the eradication of the remaining few cells by slightly improved drug treatment would produce cures in this tumor model. The efficacy of this drug when administered by iv route was also demonstrated against both ip (table 2) and iv (table 3) tumor implants. Thus, the potent antileukemic activity of ara-AIPy against both P388 and L1210 leukemia models suggests a possible future role for this drug in the treatment of human neoplasms.

Similarly, ara-AIPy significantly inhibited the growth of mammary adenocarcinoma 755 (TWI, 82%) and Lewis lung carcinoma (TWI, 68%) (tables 4 and 5). These data indicate that ara-AIPy is also very active against murine solid tumors. Hori et al (15) recently and Hoshi et al (12) earlier have reported the growth-inhibitory effects of ara-C and its derivatives on the growth of mammary adenocarcinoma 755 and pulmonary metastasis of Lewis lung carcinoma. The growth inhibition reported with ara-C in their studies seems to support the results obtained with ara-AIPy in the present report. We envisage that ara-AIPy due to its deaminase-resistant nature may further impart the desired pharmacological distribution and thus be able to inhibit the growth in a metastatic disease state.

In summary, these data and the results of our previously published reports (19,20) show that ara-AIPy is a water-soluble, deaminase-resistant, depot-form of ara-C with profound and broad-spectrum antitumor activity. The therapeutic effectiveness of this agent in transplantable animal tumor models compares favorably with ara-C or any of its known derivatives. However, it remains to be determined (*a*) whether the antitumor properties associated with ara-AIPy are solely due to its slow conversion to ara-C resulting in a pharmacological advantage by maintaining the effective serum levels of ara-C and its cytotoxic metabolites over a prolonged period, or (*b*) whether this derivative, at least partly, is directly metabolized by deoxycytidine kinase and thus exerts the growth-inhibitory effects independently. In view of the reported phosphorylation of anhydro-ara-C by deoxycytidine kinase (24), it is reasonable to assume that ara-AIPy may be a substrate for this enzyme. The lack of antitumor activity of this drug against L1210/ara-C (20) does not rule out this mechanism as a mode of its activation because the ara-C-resistant mutants are known to be devoid of kinase activity. It is thus envisaged that both of these mechanisms may be operative and contribute to the biological effects of this derivative. These questions will be addressed with the utilization of recently synthesized radiolabeled drug in our laboratory and the results will be the focus of a future report.

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Multivesicular Liposomes Containing Cytarabine for Slow-Release Sc Administration[1,2]

Sinil Kim* and Stephen B. Howell[3]

Sc administration of cytarabine entrapped in multivesicular liposomes to BDF_1 mice resulted in slow first-order release from skin, with a half-life of 4 days. The half-life was 10 minutes for unencapsulated free drug. A single-dose sc treatment of BDF_1 mice, inoculated iv with 10^5 L1210 leukemia cells 24 hours previously, resulted in long-term survivors over a wide dose range. This study demonstrates that encapsulation of cytarabine in multivesicular liposomes results in slow release from skin and that a single sc dose is often curative for mice with systemic leukemia. [Cancer Treat Rep 71:447-450, 1987]

Cytarabine is a highly schedule-dependent anticancer drug. Because this drug kills cells only when they are making DNA, a prolonged exposure at therapeutic concentration of the drug is required for optimal cell kill. Unfortunately, the half-life of cytarabine after an iv or sc dose is very short. At present, the only way of achieving a prolonged plasma level is through continuous iv or sc infusion, both of which are inconvenient and costly. Therefore, an acceptable slow-release depot preparation of cytarabine is needed. In the past, investigators have attempted to achieve this by chemical modification of the drug molecule to retard metabolism or covalent attachment of a hydrophobic moiety to retard solubilization. Such manipulations have resulted in new toxic effects (1) or unacceptable pharmacokinetic or formulation problems (2). An attempt to produce a depot preparation by suspending crystals of cytarabine in an arachis oil with aluminum distearate failed in both rabbits and humans (3).

We have studied the pharmacokinetics and efficacy of cytarabine entrapped in multivesicular liposomes in the L1210 model. Multivesicular liposomes (4) are microscopic particles composed of bilayer lipid membranes that enclose multiple noncentric aqueous chambers. This type of liposome has advantages over unilamellar (5) liposomes in that the drug molecules in inner chambers have to diffuse through multiple lipid membranes to reach the outside, resulting in better stability and control over leakage. In addition, the internal aqueous space is large relative to the space taken up by the thickness of lipid membranes, resulting in more efficient entrapment of water-soluble molecules than can be achieved with multilamellar liposomes (6).

MATERIALS AND METHODS

Materials

Cytarabine (Cytosar-U) was a gift from Upjohn, Kalamazoo, MI; dioleoyl lecithin, dipalmitoyl phosphatidylglycerol, and cholesterol were purchased from Avanti Polar-Lipids, Inc, Birmingham, AL; triolein and L-lysine, free-base, were procured from Sigma, St. Louis, MO; and nanograde chloroform was from Mallinckrodt, Paris, KY. They were all used without further purification. [^{3}H]cytarabine was purchased from Amersham. Arlington Heights, IL, and the purity was checked by high-performance liquid chromatography. BDF_1 and DBA/2 mice were from Simonsen Laboratories, Gilroy, CA; the vortex mixer was from American Scientific Products, Catalogue #S8223-1, McGaw Park, IL.

Synthesis of Multivesicular Liposomes

For each batch of liposomes prepared, 1 ml of cytarabine solution in water (20 mg/ml), with pH adjusted to 1.1 with 1 N hydrochloric acid, was added into a 1-dram vial containing 9.3 μmols of dioleoyl lecithin, 2.1 μmols of dipalmitoyl phosphatidylglycerol, 15 μmols of cholesterol, 1.8 μmols of triolein, and 1 ml of chloroform. The vial was attached to the head of the vortex mixer and shaken at the maximum speed for 6 minutes. Each half of the resulting "water-in-oil" emulsion was individually

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squirted rapidly through a narrow-tip Pasteur pipette into 1-dram vials, each containing water (2.5 ml), glucose (3.2 g/100 ml), and free-base lysine (40 mM), and then shaken on the vortex mixer for 3 seconds at "5" setting to form chloroform spherules. The chloroform spherule suspensions in the two vials were transferred into the bottom of a 250-ml Erlenmeyer flask containing water (5 ml), glucose (3.2 g/100 ml), and free-base lysine (40 mM). A stream of nitrogen gas at 7 L/minute was flushed through the flask to evaporate chloroform over 10–15 minutes at 37°C. The liposomes were then isolated by centrifugation at 600 × *g* for 5 minutes and washed with 0.9% NaCl solution. The liposomes were used within 24 hours.

Drug-Release Studies

Cytarabine release studies were done with tritium-labeled drug by adding at least 100 volumes of phosphate-buffered saline or blood-bank plasma to the washed liposome pellets. The liposome suspension was placed in a syringe, 0.01% sodium azide was added to inhibit growth of micro-organisms, air was excluded from the syringe to reduce oxidation of lipids, and the syringe was placed on a rotary shaker (G10 Gyrotory Shaker, New Brunswick Scientific Co, NJ) in a warm room kept at 37°C. At appropriate time points, aliquots were removed from the syringe, liposomes were separated from the suspending medium by centrifugation in Eppendorf microfuge for 5 minutes, and the radioactivity in the liposome pellet was compared against that at time zero and expressed as the percentage remaining in liposomes. At time zero, 96% ± 2.7% of the radioactivity was recovered in the pellet. For the release studies in 100% human plasma, liposomes were separated from the suspending medium by ultrafiltration through a YMT membrane (Amicon Corp, product #4104, Danvers, MA) because the liposomes floated up in plasma. The radioactivity in the ultrafiltrate was compared against that in an equal volume of prefiltration plasma to calculate the percentage of cytarabine released. The pH of plasma did not change significantly during the leakage studies.

In Vivo Studies

Sc pharmacokinetic studies were done on male BDF_1 mice weighing 20–25 g. A group of mice were injected sc with 1.1 mg of free or encapsulated drug in 0.1 ml of 0.9% NaCl solution with 5/8-inch, 30-gauge hypodermic needles in the middle of the abdominal skin. Such sc injections reproducibly produced immediate sc induration at the needle tip unless the needle penetrated into the peritoneal cavity. Care was taken to minimize backleakage through the needle hole by tunneling sc as far as possible before injecting. At appropriate time points, animals were killed by cervical dislocation. The full thickness of the abdominal wall tissue, including the entire skin and the underlying peritoneal membrane, was excised from the costal margin to the inguinal area from one flank to the other. The specimens were kept frozen at −20°C until analysis.

The entire specimen was homogenized after addition of at least 20 ml of distilled water with a Polytron homogenizer (Brinkmann Instruments, Westbury, NY) in the presence of 10 μM tetrahydrouridine. The homogenate was sonicated for 60 seconds with a probe sonicator (maximum setting on Biosonik IV, VWR Scientific, San Francisco, CA) and filtered through a YMT ultrafiltration membrane (Amicon Corp, product #4104). The ultrafiltrate was analyzed with a Waters and Associates (Milford, MA) high-performance liquid chromatographic scan with 280-nm detector as previously described (7) on a C18 reverse-phase Radial-Pak cartridge column, with 5 mM potassium phosphate buffer at a pH of 7.0 as mobile phase running at a rate of 2.0 ml/minute. Retention time for cytarabine was 5 minutes and that for the major metabolite, uracil arabinoside, was 7 minutes.

For toxicity and efficacy studies, BDF_1 mice were inoculated with 10^5 L1210 cells iv into the tail vein on Day 0 and treated sc with liposomes suspended in 0.9% NaCl solution on Day 1. The amount of cytarabine in liposomes was measured on a spectrophotometer (UVIKON 810, Kontron International, Zurich) at 280 nm in 100% methanol containing 10 mM sodium acetate. The absorbance was linear with respect to concentration in the range measured. The liposome suspension in 0.9% NaCl was diluted to contain 10 mg/ml of cytarabine and appropriate volumes were injected for various dosage levels. When a > 1-mg dose/mouse was given, multiple injections were done at several sites so that ≤ 1 mg/site was given.

RESULTS

Characterization of multivesicular liposomes containing cytarabine.—The average volume-adjusted size (4) of the liposomes (± SD of the distribution) was 19.4 ± 6.5 μm. Percentage of capture was 59% ± 7% (n = 12) and capture volume was 36 ± 4 μl/mg of total lipids used. The half-lives of drug leakage in phosphate-buffered saline and 100% human plasma were 18 and 12 days, respectively.

Subcutaneous pharmacokinetics.—Following sc injection of free cytarabine, the total amount of drug in the skin decreased exponentially, with a half-life of 10 minutes (fig 1). The drug encapsulated in multivesicular liposomes escaped from the injection site, with a half-life of 4 days (fig 1). Analysis of blood showed undetectable levels of cytarabine and the major metabolite uracil arabinoside from the mice injected with the liposome-entrapped drug (limits of detection, 0.25 μg/ml).

Efficacy studies.—Figure 2 shows the survival curves for mice bearing iv inoculated L1210 leukemia treated sc with varying single doses of cytarabine. Two of five animals treated with 60 mg/kg of cytarabine in liposomes survived > 60 days, four of five treated with 140 mg/kg survived long term, and four of five given 210 mg/kg died of toxicity in 6–8 days. Unencapsulated free

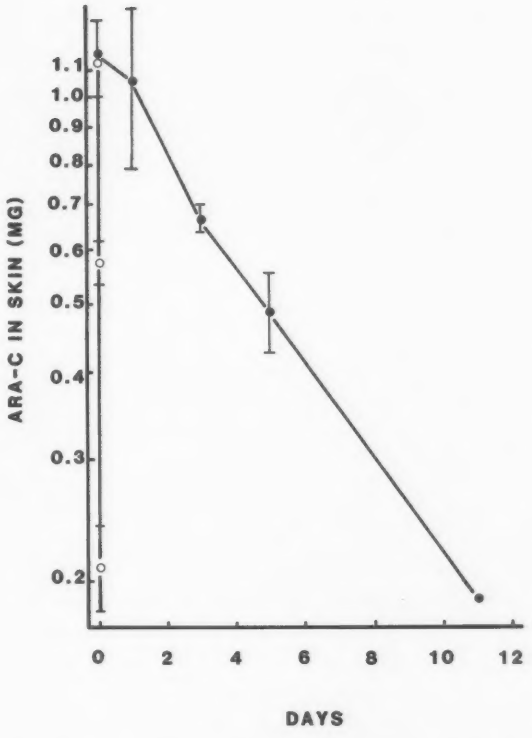

FIGURE 1.—Pharmacokinetics of cytarabine remaining at injection site after sc injection of 1.1 mg of unencapsulated "free" drug (○) or liposome-encapsulated drug (●). Each point represents the mean and SD of results from 3 mice except for the Day 11 point.

drug, given at a nontoxic dose of 210 mg/kg, resulted in no significant extension of life.

DISCUSSION

Since cytarabine is lethal to cancer cells only when they are making DNA, the drug must be present in the environment of the cancer cells for a sufficient period of time so that all or most of the cancer cells have attempted to divide at least once for optimal cell kill. Because cytarabine has a very short half-life after iv or sc injection, the drug is usually given as a continuous infusion, which is inconvenient. We have shown in an animal model that sc administration of cytarabine entrapped in multivesicular liposomes results in extremely slow release of the drug from the skin. Although we have not been able to detect cytarabine in the plasma because the released drug is metabolized so quickly, we have shown, through its biologic effect, that there is a therapeutic concentration of drug present systemically for a sufficient duration to cure the mice of L1210.

There are two likely mechanisms of drug release from the injection site. The first mechanism is leakage of drug from liposomes and absorption of the free drug into the systemic circulation. This route of drug release would be identical to the constant sc infusion. The second mechanism is the escape of whole intact liposomes in toto into the lymphatic channels, which would eventually empty into systemic circulation. While in the lymphatics, a portion or all of the drug in the liposomes may be released into the lymph fluid. Investigators in the past have explored selective intralymphatic treatment using sc injected liposomes (8,9), and a significant fraction of liposomes was found to be delivered to the lymphatics. The liposomes used, however, were of different

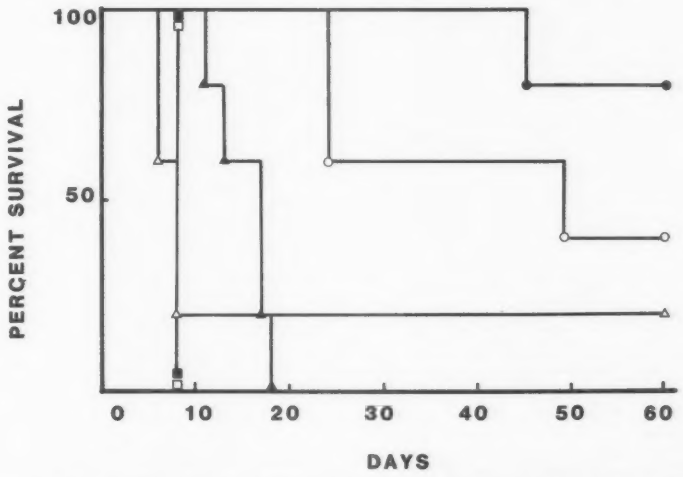

FIGURE 2.—Therapeutic effects of cytarabine in multivesicular liposomes. Mice were inoculated with 10^5 L1210 cells iv into tail vein on Day 0 and treated with a single sc dose on Day 1. Doses of cytarabine in liposomes were 213 mg/kg (△), 140 mg/kg (●), 60 mg/kg (○), and 33 mg/kg (▲). Controls were no drug (■) and free drug at 213 mg/kg (□).

type and size (eg, multilamellar liposomes, 0.02–5 μm in diameter) than those used in the present study, and therefore the data cannot accurately predict the behavior of multivesicular liposomes. Although we have not studied the intralymphatic clearance of sc administered multivesicular liposomes, the question of intralymphatic treatment would be worth investigating in an animal model of local intralymphatic tumor.

In view of the pharmacokinetics and efficacy of a single sc dose of multivesicular liposomes containing cytarabine in an animal model, we believe that such a formulation may be useful in the treatment of myelodysplastic syndromes, acute leukemias, or lymphomas. It may be particularly useful in the development of outpatient treatment programs.

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Phase II Study of Idarubicin Given Orally in the Treatment of Anthracycline-Naive Advanced Breast Cancer Patients[1]

Lars Bastholt[2,*] and Mads Dalmark[3]

A phase II study of 4-demethoxydaunorubicin (idarubicin) administered orally (45 mg/m²) every 3 weeks was conducted in patients with anthracycline-naive advanced breast cancer. Fifty patients were eligible to enter the trial; the median time to disease progression for all 50 patients entered was 22 weeks. Four patients achieved complete response and 14 achieved partial response. Response rate was 36% (complete response + partial response) for all patients entered. The median duration of tumor regression was 39 weeks. The treatment toxic effects observed were both hematologic and nonhematologic and of moderate degrees. Hair loss was observed in 86% of the patients, nausea and vomiting in 96%, and diarrhea in 36%. Cardiac function was monitored with isotope angiocardiography and no patients developed clinical congestive heart failure, up to a median cumulative dose of 260 mg/m². The left ventricular ejection fraction decreased with a median value of 4% in seven patients treated with > 360 mg/m². This study indicates that idarubicin administered orally has pharmacodynamic effects comparable to those of doxorubicin administered iv on an every-3-week schedule. [Cancer Treat Rep 71:451-454, 1987]

The anthracyclines, especially doxorubicin, are cytostatic antibiotics which have proven to be beneficial in the treatment of many solid tumors. However, there are side effects including the risk of cardiomyopathy, pronounced vomiting, hair loss, tissue necrosis by accidental extravasation, and staff expenses for iv administration of the drugs. During the last decade continuous laboratory research has been carried out to identify analogs overcoming these side effects and still having at least identical antitumor activity.

Idarubicin (DMDR) (1) is a new analog of daunorubicin which has shown activity orally (2). Acute and chronic toxic effects following DMDR were similar to those of the parent compound, but cardiac toxicity in rabbits and mice was comparatively lower. This indicates that DMDR may have a better therapeutic index (3). The recommended starting dose of DMDR administered orally in phase II studies has been estimated to be between 45 and 50 mg/m² every 3 weeks (4). Preliminary results of this work have been published earlier as a congress abstract (5).

MATERIALS AND METHODS

Patients entering this trial had histologic proof of their primary breast cancer, and they had progressive and measurable/evaluable advanced disease. Lymphedema, hilar enlargement, pleural effusion, marrow depression, and osteoblastic skeletal lesions were not considered evaluable lesions. Prior hormonal treatment was allowed provided that there was at least a 4-week interval from the last day of endocrine therapy. Previous chemotherapy was allowed provided it was not an anthracycline-containing combination and had been stopped for a minimum of 6 months. Patients with clinical signs of congestive heart failure or a history of prior myocardial infarction were excluded from the trial. Patients with central nervous system metastases and patients with wbc count $< 3.0 \times 10^9$ cells/L, platelet count $< 100 \times 10^9$ cells/L, or bilirubin > 50 μmols/L were excluded.

Hemoglobin, leukocytes, and platelets were measured prior to start of treatment on Days 14 and 21 and thereafter every 3 weeks at scheduled re-treatment time. Patients with soft tissue metastasis were evaluated by physical examination every 3 weeks. Patients with bone or visceral metastases were evaluated with x-ray investigations, ultrasound, or computerized tomographic scans every 3 months. Informed consent was obtained according to the Helsinki declaration.

The standard dose of DMDR was 45 mg/m² orally every 3 weeks. Patients with bilirubin between 35 and

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TABLE 1.—Characteristics of 50 patients with advanced breast cancer treated with DMDR orally

Characteristic	No. of patients (%)
Menopausal status	
Premenopausal	1
Postmenopausal	49
Median age in yrs (range): 69 (42–78)	
Median performance status (range): 1 (0–3)	
Dominant lesion	
Visceral	17 (34)
Osseous	17 (34)
Soft tissue	16 (32)
Prior treatment	
Mastectomy	48 (96)
Radiotherapy	39 (78)
Endocrine therapy	40 (80)
Chemotherapy	
Adjuvant	1 (2)
Advanced disease	2 (4)

50 μmols/L received a 50% reduced starting dose. At least 310 of 333 courses (93%) were administered in the outpatient clinic superintended by a nurse. The drug was given together with a light meal and under cover of standard antiemetics (metoclopramide or metopimazine). Treatment was postponed by 1 week if there was not full hematologic recovery (wbc count $> 3 \times 10^9$ cells/L; platelet count $> 100 \times 10^9$ cells/L) from the prior course at scheduled re-treatment time, Subsequently, the dose was adjusted as follows. The dose was reduced to 50% if the wbc counts were between 2 and 3 $\times 10^9$ cells/L, and/or the platelet counts were between 75 and 100 $\times 10^9$ cells/L, and/or the bilirubin was between 35 and 50 μmols/L. In case of lower values of either wbc counts and platelet counts or *higher* bilirubin, dose zero was given. In case of no response or toxicity, dose was escalated by 5 mg/m^2 every second course.

Treatment was discontinued if the patient refused treatment or if disease progression or severe toxicity occurred. It was recommended not to exceed a maximum cumulative dose of 320 mg/m^2 of DMDR, but it was left to the responsible investigator to continue treatment past this limit under careful cardiac monitoring.

World Health Organization (WHO) criteria (6) for reporting results of cancer treatment were used for grading the pharmacodynamic effects of DMDR. All relevant data on responding patients [partial response (PR), complete response (CR)] were submitted to second-party review, whenever the study monitors assumed an antitumor activity of DMDR to be demonstrable. The second-party reviewer decided autocratically whether or not the patients had responded and the duration of response. The latter was measured for both PR and CR from the first day of treatment to the date of first observation of progressive disease.

The cardiac function was monitored by clinical examination, chest x-ray, and isotope angiocardiography. Cardiac ejection fraction was estimated prior to start of treatment and every 3 months.

RESULTS

Response

Characteristics of the 50 eligible patients are shown in table 1. All patients had complete follow-up. Fifty-one patients were formally registered for entry in this study between November 1983 and July 1985. One patient was considered to be ineligible because of central nervous system metastases actually demonstrated before the start of DMDR treatment. All data on this patient were deleted from the following report. Six of 50 eligible patients did not receive more than one treatment (table 2). This was due to death from a cerebral stroke (with normal platelet count) (one patient), refusal of further treatment because of severe gastrointestinal toxicity (two), and rapidly progressive disease (three).

Forty-four patients received at least two cycles of DMDR. The median time from the first day of treatment to the date of first observation of progressive disease for all 50 eligible patients treated with DMDR was 22 weeks (95% exact confidence limits, 15–32).

Four patients achieved CR, 14 achieved PR, 19 had no change, and seven had progressive disease (table 2). The overall response rate (CR + PR) for all 50 patients treated at least once with DMDR was 36% (95% exact confidence limits, 23%–51%). The overall response rate for 44 patients treated at least twice with DMDR was 41% (95% exact confidence limits, 26%–57%).

The median duration of tumor regression (CR + PR)

TABLE 2.—Overall response to DMDR administered orally

		Response*					
	No. of patients	CR	PR	NC	PD	NE	Too early
After 1 course of therapy	50	—	—	—	3	1†	46‡
After 2 courses of therapy	44	4	14	19	7	—	—

*NC = no change; PD = progressive disease; and NE = not evaluable.
†1 patient died from a cerebral stroke after 1 course.
‡2 patients refused further treatment because of gastrointestinal toxicity.

for the 18 responding patients from the first day of treatment to the observation of progressive disease was 39 weeks (95% exact confidence limits, 29–46).

The overall response rate in relation to the dominant site of disease was 62% (ten responses among 16 patients) in patients with soft tissue disease, 18% (three responses among 17 patients) in patients with dominant visceral disease, and 29% (five responses among 17 patients) in patients with dominant disease in bones.

Toxicity

Hematologic toxicity was evaluated on Day 14 following the first dose where 49 patients received 45 mg/m^2 of DMDR. One patient heavily pretreated with radiotherapy received a starting dose of 30 mg/m^2. The median nadir leukocyte count, platelet count, and hemoglobin were 2.2×10^9 cells/L (range, 0.3–10.5), 178×10^9 cells/L (range, 10–439), and 7.5 mmols/L (range, 3.6–8.9), respectively, on Day 14.

Among treatment-related nonhematologic toxicity, nausea and vomiting was observed in 48 of 50 patients (96%), with a median WHO grade of 2. Diarrhea was observed in 18 of 50 patients (36%), with a median WHO grade of 2. Hair loss was observed in 42 of 49 patients (80%), with a median WHO grade of 2.

No patient developed clinical congestive heart failure. Isotope angiocardiography showed a median starting value of left ventricular ejection fraction (LVEF) of 64% (range, 42%–82%). The relationship between the cumulative dose of DMDR and median value and range of the absolute changes in LVEF from the starting values is shown in table 3.

DISCUSSION

DMDR is a new anthracycline analog which is currently being studied in an effort to identify anthracyclines with a better therapeutic index.

A few reports have appeared on the therapeutic activity of DMDR administered orally every 3 weeks in women with advanced breast cancer. The results indicate that a response rate of 35%–40% is obtainable in previously untreated patients compared to a response rate between 20% and 30% in previously treated patients (7,8).

A median leukocyte count nadir of 2.2×10^9 cells/L shows that moderate hematologic toxicity was obtainable using a starting dose of 45 mg/m^2. Median duration of response in this study was 9 months (39 weeks). This value is comparable to published data, where median durations between 4 and 6.7 months were reported in previously untreated breast cancer patients receiving doxorubicin monotherapy on a 3-week schedule (9–11).

TABLE 3.—Relationship between cumulative dose of DMDR and median value/range of the absolute changes in LVEF from the initial values

Cumulative dose of DMDR (mg/m^2)	No. of patients	Median value (%)	Range
< 180	26	−2	+11 to −14
180–360	23	−3	+10 to −10
360–540	7	−4	+3 to −8
540–720	4	−2.5	+2 to −11
> 720	1	−5	

Published data concerning the response rate in this patient group vary between 38% (12) and 47% (11), and these data are comparable to the response data presented in this study (36%). With regard to hematologic and nonhematologic toxicity, the pattern of doxorubicin toxicity is comparable to our observations with DMDR (11).

No definitive statements can be made regarding the possible lower cardiotoxicity of DMDR but we observed no clinical signs of congestive heart failure, and the changes in LVEF were minimal throughout the treatment. The changes we found in LVEF throughout treatment were comparable to what has been shown in patients treated with doxorubicin (13).

In conclusion, the pharmacodynamic effects of DMDR given orally were comparable to those of doxorubicin given iv on an every-3-week schedule. However, patient compliance is an important factor when administering drugs orally. The nonhematologic toxicity of the present schedule with DMDR may compromise patient compliance. Most patients, however, do prefer oral treatment to iv treatment and a search for a treatment schedule with less toxicity is warranted.

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High-Dose-Intensity Regimen of Weekly Doxorubicin and Cisplatin in the Treatment of Patients With Stage III and IV Epithelial Ovarian Carcinoma[1,2]

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Thirty-seven eligible patients with stage III or IV epithelial ovarian carcinoma were treated with weekly doxorubicin and weekly escalating cisplatin in an attempt to improve outcome by increasing the dose intensity of chemotherapy as compared to more conventional regimens given every 3–4 weeks. There was a 63% clinical response rate, with a median survival time of 18 months. Six patients (16.2%) had a complete surgical/histologic response (negative second-look laparotomy). Two patients had treatment-related deaths, and severe toxicity was responsible for four other patients having to discontinue treatment prematurely. The toxicity of this regimen jeopardized received (as opposed to projected) dose intensity and the regimen therefore offered no improvement compared to other drug combinations and schedules. [Cancer Treat Rep 71:455–458, 1987]

Since the introduction of cisplatin to the treatment of advanced ovarian carcinoma, response rates have improved (1–3), but if there has been any impact on long-term survival at all, it has been small, with cure rates remaining disappointingly low. The search therefore continues for improved first-line therapy.

Most treatment regimens include cisplatin at a dose of 50–75 mg/m^2 every 3–4 weeks for six to nine courses, with the total dose being approximately 450 mg/m^2. In 1981, one of the investigators (G. O'Connell) initiated a pilot study of weekly and escalating doxorubicin and cisplatin to a total dose of 460 mg/m^2 of each drug over a period of approximately 4 months. It was hypothesized that increased treatment frequency might increase efficacy and might also decrease the gastrointestinal toxicity of cisplatin and decrease the risk of doxorubicin cardiotoxicity (4).

In this first pilot study, eight patients were treated with this regimen and five had histologically negative second-look laparotomies (5), for a complete surgical/histologic response rate of 63%. The National Cancer Institute of Canada Ovarian Carcinoma Group was impressed with the results in this small sample but felt that the response rate should be confirmed in a larger group. It was also felt that the feasibility of a weekly dose schedule and its toxicity should be assessed in a number of cancer treatment centers. This weekly regimen was therefore used in the treatment of 37 patients with advanced ovarian carcinoma treated in five Canadian cancer treatment centers. The results are reported in this paper. The dose of cisplatin was escalated in the same way as it was in the first pilot study but it was decided not to escalate the doxorubicin because its escalation had caused considerable toxicity necessitating dose modifications and treatment delays of both drugs; it was hoped that, by maintaining a constant doxorubicin dose, these problems could be avoided.

PATIENTS AND METHODS

Between August 1983 and May 1984, 42 patients were entered in this study. To be eligible, patients had to have International Federation of Gynecology and Obstetrics stage III or IV epithelial ovarian carcinoma that had not been previously treated. Bone marrow function had to be adequate, with a neutrophil count > 1000/mm^3 and a platelet count > 100,000/mm^3. Patients were excluded if they had renal or hepatic impairment (BUN > 30 mg/dl; serum creatinine > 1.5 mg/dl; bilirubin > 5 mg/dl; or SGOT > 600% of upper normal) or if they had serious heart disease. They were not excluded on the basis of age or performance status. The World Health Organization histologic classification (6) and the pattern grading system (7,8) were used and all pathology was

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reviewed by one pathologist (M. E. Kirk). Patients with tumors of borderline malignancy were excluded.

Of the 42 patients entered, five were considered initially ineligible: two had stage IIB disease, one had a mixed mesodermal tumor, one had a tumor of borderline malignancy, and one had benign disease. The characteristics of the remaining 37 patients are shown in table 1.

Treatment was initiated within 21 days of surgery and consisted of doxorubicin at a dose of 20 mg/m^2 iv weekly and cisplatin starting at a dose of 22 mg/m^2 iv weekly. The cisplatin dose was escalated by 2 mg/m^2 each week until the dose of 30 mg/m^2 was reached, provided that the serum creatinine was not $>$ 1.5 mg/dl. Treatment was given with hydration but diuretics were not used. Treatment was continued until the total dose of cisplatin given was 460 mg/m^2, progressive disease developed, or toxicity precluded continuing treatment. Patients also received prophylactic cotrimoxazole throughout treatment. Doxorubicin was discontinued if there was any evidence of congestive heart failure, cardiomegaly, persistent cardiac arrhythmia, or a $>$ 40% loss of voltage in the six limb leads on ECG. Cisplatin was delayed if the serum creatinine was $\geq$ 1.5 mg/dl and resumed once it fell below this level. Dose modifications for hematologic toxicity and for impaired hepatic function are shown in tables 2 and 3.

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total	37
Median age in yrs (range): 60 (25–80)	
Stage	
III	27
IV	10
Greatest diameter of residual disease (cm)	
Microscopic	2
$<$ 2	10
2–10	17
$>$ 10	8
Performance status*	
0	3
1	14
2	12
3	6
4	2
Histology	
Serous	20
Mucinous	0
Endometrioid	3
Clear cell	1
Mixed	5
Undifferentiated	6
Unclassified adenocarcinoma	2
Grade	
1	1
2	12
3	23
Ungraded	1

*Eastern Cooperative Oncology Group criteria.

TABLE 2.—Chemotherapy dose modifications: hematologic toxic effects

Neutrophil count (cells/mm^3)		Platelet count (cells/mm^3)	Doxorubicin dose (%)	Cisplatin dose (%)
$\geq$ 1000	and	$>$ 100,000	100	100
500–999			65	100
		50,000–100,000	50	100
$<$ 500	or	$<$ 50,000	0	0

Prior to entry in the study, all patients underwent laparotomy for staging and maximal tumor debulking and for accurate measurement and description of residual tumor. Total abdominal hysterectomy, bilateral salpingo-oophorectomy, and omentectomy were done when possible. Initial investigations included a complete hemogram, serum BUN, creatinine, SGOT, alkaline phosphatase and bilirubin, chest x-ray, ECG, and physical examination. Hemogram and serum creatinine were done weekly prior to each treatment and biochemical profile and physical examination were done at least every 3 weeks.

Complete clinical response was defined as the complete disappearance of all cancer on physical and/or radiological examination. Partial clinical response was defined as a $\geq$ 50% decrease in the sum of the products of the perpendicular diameters of bidimensional lesions or a $\geq$ 50% decrease in the linear tumor measurement of unidimensional lesions as assessed on physical or radiological examination. Progressive disease was defined as a $\geq$ 50% increase in the size of any lesion or the development of any new lesion. No change was defined as an increase or decrease in tumor volume of $<$ 50%.

If patients had no clinically detectable disease present at the completion of treatment, they underwent a second-look laparotomy to assess surgical histologic response. Second-look laparotomy included washings for cytology and multiple peritoneal biopsies including the diaphragmatic surfaces and abdominal gutters, pelvic peritoneum, and serosa of small and large bowel. Additional sampling of any residual omentum and/or pelvic and para-aortic nodes was performed. If there was no gross evidence of residual cancer at surgery and all of the above biopsies and washings were negative, the patient was considered to have had a complete surgical/histologic response (negative second-look). A partial surgical/histologic response was defined as a reduction $\geq$ 50% in the total tumor burden as compared to the findings at initial surgery. No change and progressive disease were defined as above, again as compared to the findings at initial surgery.

TABLE 3.—Chemotherapy dose modifications: hepatic toxic effects

Bilirubin (mg/dl)	Doxorubicin dose (%)
$<$ 1.5	100
1.5–3.0	50
3.1–5.0	25
$>$ 5.0	0

TABLE 4.—Clinical response*

Response	No. of patients (%)
Patients with no clinically detectable disease initially	
Total	13
Disease-free at treatment completion	13
Progressed on treatment	0
Patients with clinically detectable disease initially	
Total	24
Complete clinical	5 (21)
Partial	10 (42)
No change	2 (8)
Progressive disease	7 (29)

*Total No. of patients overall who progressed on treatment, 7 (19%).

RESULTS

Antitumor Effects

The results regarding clinical response are shown in table 4. Of the 37 eligible patients, 13 (35%) had no clinically detectable disease at the completion of their initial surgery and prior to therapy. They are therefore not evaluable for clinical response. All of these 13 patients remained clinically free of disease at the completion of chemotherapy. Of the remaining 24 patients (65%) who did have clinically detectable disease at the start of treatment, five (21%) had complete response, ten (42%) had partial response, two (8%) had no change, and seven (29%) developed progressive disease on treatment. When all patients are considered, seven (19%) of the 37 patients developed progressive disease on treatment.

At the completion of treatment, 18 patients had no clinically detectable disease (13 who had no clinically detectable disease initially and five who had a complete clinical response). One patient, who had no clinically detectable disease initially, discontinued treatment because of toxicity after only four treatments and she is therefore considered a treatment failure. The remaining 17 patients had second-look laparotomy and the results are shown in table 5. Six of the 17 patients (35%) had complete surgical/histologic response (negative second-look), eight (47%) had partial response, and three (18%) had no change. Thus, of the total group of 37 patients, six had complete surgical/histologic response, giving an overall negative second-look rate of 16.2%. Five of these six patients presented with stage III disease and one presented with stage IV disease (pleural fluid positive for malignancy). One of these patients had residual tumor > 10 cm in diameter at the start of treatment, one had tumor 2-10 cm in diameter, two had visible tumor < 2 cm in diameter, and one had only microscopic residual tumor following initial surgery prior to the start of treatment.

TABLE 5.—Surgical/histologic response: results of second-look laparotomy*

Response	No. of patients (%)
Clinically disease-free at treatment completion	18
Underwent second-look laparotomy	17
Complete surgical/histologic	6 (35)
Partial surgical/histologic	8 (47)
No change	3 (18)
Progressive disease	0

*Overall complete surgical/histologic response rate (negative second-look rate), 16.2% (6 responses among 37 patients).

Toxic Effects

There were two deaths attributed to treatment in this study. One patient received three courses of treatment and then died of congestive heart failure, which was felt to be due to treatment-associated hydration rather than doxorubicin toxicity. The other patient developed nonketotic hyperosmolar coma with sepsis after 14 courses of treatment. The patient's serum creatinine was mildly elevated. The platelet count fell to 4000/mm^3, at which time the patient had a gastrointestinal bleed. She then developed disseminated intravascular coagulation and multisystem failure resulting in death.

Four additional patients had to discontinue all treatment early because of toxicity. Two patients developed profound, prolonged pancytopenia, both after ten courses of treatment. One of these patients developed sepsis and the other also had mild elevation of the serum creatinine and congestive heart failure. Neither patient resumed treatment once the blood cell counts returned to normal. Another patient was hospitalized due to anorexia, dehydration, and severe weight loss after four courses of treatment. The fourth patient had had seven courses of

TABLE 6.—Toxicity

Toxic effect	No. of patients (%)
Leukopenia (× 10^3 cells/mm^3)*	
Wbc count 1.0 to < 2.0 and/or neutrophil count 0.5 to < 1.0	8 (22)
Wbc count < 1.0 and/or neutrophil count < 0.5	23 (62)
Thrombocytopenia (× 10^3 cells/mm^3)*	
50 to < 90	2 (5)
25 to < 50	8 (22)
< 25	4 (11)
Gastrointestinal	
Nausea only	7 (19)
Nausea and vomiting, controllable	11 (30)
Intractable vomiting	8 (22)
Stomatitis	
Soreness	3 (8)
Ulcers, can eat	5 (14)
Ulcers, cannot eat	6 (16)
Renal	
Creatinine (mg/dl)	
1.3–2.0	10 (27)
2.1–4.0	2 (5)
> 4.0	1 (3)
Cardiac	4 (11)
Infection	3 (8)
Peripheral neuropathy	1 (3)

*Nadir count during total treatment.

doxorubicin in 9 weeks but only three courses of cisplatin because of mild elevation of the serum creatinine. The neutrophil count was also persistently low, resulting in a minimum dose of doxorubicin being given; because only such small amounts of drug were able to be administered, the treatment was changed.

In addition to the two patients described above who developed sepsis, there was only one other reported case of infection and this was the development of an abscess at the colostomy site of one patient while she had neutropenia. The infection subsided with treatment and chemotherapy was continued.

In addition to the two patients described above who developed congestive heart failure, one other patient developed heart failure after 155 mg/m^2 of doxorubicin. All treatment was discontinued at that time because of progressive disease. The heart failure resolved. Another patient discontinued doxorubicin after 144 mg/m^2 because of the development of premature ventricular contractions. Cisplatin was continued.

Cisplatin had to be discontinued prematurely in four patients because of a persistent elevation of serum creatinine $>$ 1.49 mg/dl. Only one patient developed peripheral neuropathy while receiving treatment.

Toxicity data are summarized in table 6.

While the planned weekly dose of doxorubicin was 20 mg/m^2/week and of cisplatin was 28.75 mg/m^2/week, the actual median received dose of doxorubicin was 13.2 mg/m^2/week (mean, 13.5; range, 0.9–21.3) and the median received dose of cisplatin was 22.6 mg/m^2/week (mean, 21.0; range, 1.2–33.7). The median survival of this patient group was 18 months.

DISCUSSION

There is evidence from clinical data on both advanced breast cancer patients and advanced ovarian carcinoma patients that suggests that higher chemotherapy dose intensity (increased dose per unit time) might beneficially affect treatment outcome (9,10). Dose scheduling impacts on treatment outcome, probably inasmuch as it determines the received, as opposed to the predicted, dose intensity delivered. The most common dose and schedules of both doxorubicin and cisplatin used in published large series of ovarian carcinoma patients are 50 mg/m^2 given either every 3 or 4 weeks, giving predicted dose intensities of 12.5–16.7 mg/m^2/week. The projected dose intensity in this study was therefore higher than is usually given in the treatment of ovarian carcinoma, but because of its toxicity, it is possible that the received dose intensity was more comparable with received dose intensities of less toxic regimens in which dose reductions were not as severe. This may have accounted for the clinical response rate of 63% and the median survival time of 18 months, which are comparable to the results seen with conventional treatment.

Conclusions regarding the value of dose intensity cannot be made based on the results of this small patient sample, since specific patient characteristics of the group, as well as the toxicity of the weekly schedule, may well have adversely affected outcome. The toxicity experienced was marked, with two patients having treatment-related deaths and four additional patients having to discontinue all treatment prematurely because of severe toxicity.

The results of large-scale randomized trials will be required to determine the value of both the dose intensity and the total dose of effective drugs in the treatment of this disease. However, we can conclude that the weekly dose and schedule of doxorubicin and cisplatin that was used in this trial is unacceptably toxic and does not improve survival when compared to less frequently delivered treatment and it therefore should not be recommended in the treatment of ovarian carcinoma.

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Phase I–II Study of Eflornithine and Mitoguazone Combined in the Treatment of Recurrent Primary Brain Tumors[1,2]

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Eflornithine (DFMO), an irreversible inhibitor of ornithine decarboxylase, and mitoguazone (MGBG), a competitive inhibitor of *S*-adenosylmethionine decarboxylase, were evaluated in a phase I–II study for patients with primary recurrent malignant brain tumors. All patients had failed prior radiation therapy and most had also failed prior chemotherapy. Two dose schedules were used, with the second schedule (Group II) a modification of the first schedule (Group I). The Group II schedule, with different dose levels, was better tolerated than the Group I schedule. Gastrointestinal and myelotoxicity were dose-limiting in most patients, and tinnitus was dose-limiting in two patients. Nineteen of 33 evaluable patients had anaplastic gliomas, in whom response was observed in 21%, stable disease in 53%, and immediate progression after one course of therapy in 26%. Of six patients with glioblastoma multiforme, two had brief stabilization of disease. An additional patient with brainstem glioma and ependymoma also had disease stabilization. Four patients with medulloblastoma, a spinal cord mixed glioma, and one with oligodendroglioma failed DFMO-MGBG. Based on this study, we believe that a combination of DFMO and MGBG is well-tolerated and deserves further evaluation for patients with anaplastic gliomas, particularly those that appear to be biologically slow growing. [Cancer Treat Rep 71:459–464, 1987]

Eflornithine (DFMO) is an irreversible inhibitor of ornithine decarboxylase (28) and mitoguazone (MGBG) is a competitive inhibitor of *S*-adenosylmethionine decarboxylase (45). DFMO depletes both putrescine and spermidine (26,27) and, through depletion of spermidine, enhances uptake of MGBG, which is a structural analog of that compound (3,13,38,40). In cell culture and against a number of tumor models, depletion of polyamines by DFMO inhibits cell proliferation and slow tumor growth (5,11,12,25–27,33,36,37,40). MGBG, in addition to selective depletion of polyamines, inhibits RNA and DNA synthesis and mitochondrial respiration (7,16). MGBG is also cytotoxic in cell culture and against animal tumor models (29,31).

The combination of DFMO and MGBG is a logical extension from the biochemistry of polyamine synthesis. Against some tumors, the effects of DFMO and DFMO with MGBG are clearly cytocidal and usually related to depletion in constitutive polyamines (10,12,31,33,38). Against other lines, the effects appear to be more related to alteration in the tumor cell phenotype to a less transformed, or less malignant, state (15,32).

DFMO and MGBG have both been used in clinical trials as single agents (1,2,9,30,39), but the combination of DFMO and MGBG has been used in only two published phase I studies (42,44) and two anecdotal clinical reports (12,41). While some activity has been observed in leukemia (12,41) and non-small cell carcinoma, prostate carcinoma, hypernephroma, and other carcinomas (42), the combination of DFMO and MGBG has not been studied previously in patients with brain tumors.

The treatment of recurrent anaplastic astrocytomas has primarily been with the nitrosoureas such as carmustine and lomustine (19) and with procarbazine (17), alone or in combination (8,19,21). Few if any other drugs have activity (6). Because of interest in develop-

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ing new chemotherapies for brain tumors, we developed and tested a well-tolerated combination of DFMO and MGBG. The results of that study are reported here.

MATERIALS AND METHODS

Patients

All patients had unequivocal neuroradiological evidence of tumor progression at the time of treatment. Patients with a histologically verified diagnosis of glioblastoma multiforme (GM) comprised the GM group. The diagnosis of GM was based on the histologic appearance, at least focally, of a glial neoplasm with high cellularity, nuclear and cytoplasmic pleomorphism, and vascular endothelial proliferation (4). Patients with the diagnoses of highly anaplastic, moderately anaplastic, and gemistocytic astrocytoma and mixed malignant glioma constituted the anaplastic glioma (AG) group. The definitions used in histological grading of these tumors are as follows. Infiltrating highly anaplastic astrocytoma (HAA) requires that the tumor should not be a GM but must be focally moderately to highly cellular and have at least two of the following characteristics: (*a*) high nuclear/cytoplasmic ratio, (*b*) coarse nuclear chromatin, (*c*) much mitotic activity, (*d*) nuclear pleomorphism, and (*e*) cytoplasmic pleomorphism. Infiltrating moderately anaplastic astrocytoma (MOAA) is less malignant than HAA and is characterized by mild to moderately increased cellularity, enlarged nuclei, and relatively uniform cytoplasm. The term gemistocytic astrocytoma (GA) is used only when a tumor is not a GM and > 60% of the tumor cells are gemistocytes. Mixed malignant glioma (MMG) is a glioma with anaplastic features from at least two of the following tumor types: ependymoma, oligodendroglioma, and HAA or MOAA. The diagnosis of medulloblastoma was based on the histologic criteria of Russell and Rubinstein (35). Histological review was performed by R. L. Davis.

Treatment Plan

DFMO and MGBG were supplied by Merrell Dow Research (Cincinnati, OH). DFMO was used directly as an oral solution containing 1 g of active drug/5 ml of distilled water. MGBG was supplied in a sterile 1.0-g vial. When reconstituted with 9.1 ml of sodium chloride injection, USP, each milliliter contained 100 mg of MGBG at pH 3.5-5.5.

Two DFMO-MGBG schedules were used, Group I and Group II, as described below: *Group I*—DFMO given on Days 1-42 at a dose of 1.0 g/m^2 four times per day orally, and MGBG given on Days 14, 28, and 42 at a dose of 350 mg/m^2 iv over 45 minutes, and *Group II*—DFMO given on Days 1-14, 21-35, and 42-56 at a dose of 1.33 g/m^2 three times per day orally, and MGBG given on Days 14, 35, and 56 at a dose of 200 mg/m^2 iv over 45 minutes.

The Group II schedule was used more extensively because of excessive toxicity observed in our Group I patients as well as by Warrell et al (44). There were 12 evaluable patients in Group I and 21 evaluable patients in Group II.

Toxicity was graded using Northern California Oncology Group (NCOG) scales. Cbc and platelet counts were obtained every 2 weeks before the MGBG dose. Ototoxicity was evaluated by history and hearing tests every 6 months. Dose modification was made for myelotoxicity and gastrointestinal toxicity > 1 and for ototoxicity. Dose escalation of 10% was allowed initially for MGBG and in later patients for DFMO.

Evaluation of response was made 1 week after the last MGBG dose in each cycle. Courses were repeated after a 1-week break. The combination was repeated until tumor progression or until 1 year of therapy was completed.

Evaluation of Response

Evaluation of response to therapy was made using the University of California, San Francisco (UCSF) criteria (20). Prior to each course of therapy, neurological examination, computerized tomographic (CT) scan with contrast enhancement, and radionuclide (RN) brain scan were performed. Determination of response was based on sequential evaluations performed prior to each course of chemotherapy. Steroid (dexamethasone) doses were kept at the lowest value consistent with good medical practice. Responses to treatment were measured from the first dose of DFMO and were defined as follows: *response*—definite improvement (+2 score) of two of the three tests (neurological examination, CT scan, and RN scan) while receiving a stable or decreasing steroid dose; *deterioration*—definite worsening (−2 score) of two of the three tests while receiving a stable or increasing steroid dose; and *stable disease*—no definite worsening or improvement in two of the three tests.

Statistical Analyses and Data Collection

Time to tumor progression (TTP) from the initiation of therapy with DFMO was the principal endpoint of the study. For the AG group, a Kaplan-Meier curve (14) was used to calculate TTP.

RESULTS

Thirty-three of 45 patients (19 with AG, six with GM, and eight with other tumor types) entered in the study were evaluable for the determination of response to chemotherapy. There were 12 patients who were not evaluable for response or toxicity. This high percentage of nonevaluable patients was due to the phase I-II nature of the study and the fact that all 12 patients had been heavily pretreated with radiation therapy and at least one form of cytotoxic chemotherapy prior to receiving DFMO-MGBG. The specific reasons for nonevaluability are that three patients had protracted myelosuppression which prevented them from completing a course of therapy, one was unable to comply with the study, one

died of a pulmonary embolus early in the first course of therapy, and the remaining seven had been placed on the combination for "humanitarian reasons" and did not take the equivalent of half of one course of therapy; some did not even receive the first MGBG dose. These last seven patients probably should never have received the DFMO-MGBG.

Tables 1 and 2 summarize the most severe toxic effects encountered during DFMO-MGBG therapy for Groups I and II, respectively. Myelotoxicity and gastrointestinal toxicity were dose-limiting in both Groups I and II. Two patients in Group I who stabilized with therapy refused further DFMO-MGBG because of gastrointestinal toxicity. Two patients in Group II had dose reduction because of tinnitus. Hearing loss was not significant or cause for dose modification in any of the patients. Despite preclinical trials in dogs where intraventricular DFMO and MGBG were a frequent cause of seizures (22), we did not note any problems with seizure control in our patients. However, all patients were on anticonvulsants during therapy and this may have lowered any tendency for seizures with DFMO or MGBG.

Patients who were evaluable for response to chemotherapy are summarized in tables 3 and 4 for Groups I and II, respectively. If we define "benefit" from therapy as the ability to reduce the size of the tumor (responder) or maintain the tumor in a quiescent state (stable disease), then 33% of patients (two of six) with supratentorial GM and 74% (14 of 19 patients) with supratentorial AG benefited from therapy. In addition, one patient with mixed cord glioma, one with oligodendroglioma, and four with medulloblastoma showed no benefit from treatment with DFMO-MGBG. One patient with brainstem glioma and one with ependymoma showed disease stabilization for 15 and 18 weeks, respectively.

TABLE 1.—Schedule I: serious toxic effects as a function of MGBG dose

Patient No.	Average MGBG dose (mg/m^2)	No. of MGBG doses	Level of toxic effect*										
			Leukopenia	Thrombocytopenia	Hemoglobin	Nausea and vomiting	Diarrhea	Myalgia	Petechiae	Sore mouth and throat	Alopecia	Fatigue	Anorexia	Excoriation
1	170	5	2	4	4				3					
2	190	5	2	3							1	1	1	
3	145	10			4		1		1	2	2	2	2	1
4†	129	5	1	3				2						
5‡	318	6	2					3	2					
6§	367	14	1			1	4	3		3		3		
7	276	3												
8	297	7	1		4	3	4	3		2		2	1	
9¶	332	5			4			3				3	2	1
10¶	400	4						3		2			3	4
11	300	4												3
12	250	2		4									2	

*Level 1 = mild toxicity; level 2 = moderate toxicity; level 3 = severe toxicity; and level 4 = treatment discontinued or stopped for a period of time.
†Developed spinal metastasis, given concurrent cytarabine.
‡Seizure activity, unclear if drug-related.
§Documented hearing loss, unclear if radiation therapy or DFMO-MGBG induced.
¶Refused further treatment due to toxic effects.

DISCUSSION

In the current study we have shown that the combination of DFMO and MGBG is active against AG. As of November 1986 the UCSF Neuro-oncology Service had treated 19 patients with supratentorial AG using Schedules I and II and had observed four patients with responses (21%), ten with disease stabilization (53%), and five with disease progression (26%) after one course of DFMO-MGBG. Of note is that all patients had been heavily pretreated and had failed prior nitrosourea-based chemotherapy and/or radiotherapy.

Based on a Kaplan-Meier representation the median time to tumor progression (MTP) was 52 weeks for the combined responder and stable disease groups in patients with supratentorial AG. This compares favorably to previous studies with drug combination protocols using nitrosoureas and procarbazine where the MTP was 27–41 weeks for patients with AG (6,18,19). Unfortunately, DFMO-MGBG was not effective against medulloblastoma tumors.

The unusually high number of patients with AG (ten

TABLE 2.—Schedule II: serious toxic effects observed for DFMO-MGBG combination as a function of MGBG dose

Patient No.	Average MGBG dose (mg/m^2)	No. of MGBG doses	Level of toxic effect*						
			Leukopenia	Thrombocytopenia	Nausea and vomiting	Gastrointestinal	Stomatitis	Tinnitus	Hearing loss
1	200	18	2		1				
2†	200	18	1	3		2	1		1
3	199	19	2			2			
4‡	200	8	1				2	2	
5†	200	12				3	2		1
6	217	6				2			
7	200	3							
8	200	4							
9	193	15	2						
10	133	3	2						
11	151	3	2						
12	200	3							
13	200	3					2		
14	189	9	2						
15	200	3							
16	200	3							
17	210	4	2	3					
18	200	6				1			
19	200	4							
20‡	200	5	1					2	
21	200	3							

*See table 1 for definitions.
†Documented hearing loss, unclear if radiation therapy or DFMO-MGBG induced.
‡DFMO decreased because of tinnitus.

of 19) who had stable disease for substantial time periods (MTP, 50 weeks) may indicate that DFMO-MGBG altered tumor cell phenotype (16,32) rather than producing cytotoxicity.

We believe that the results presented are impressive because of the following: (*a*) we initially used MGBG as monotherapy for recurrent AG (unpublished observations on record at the National Cancer Institute), but we were unable to show activity in six patients treated at doses ≤ 600 mg/m^2/week; (*b*) since the vast majority of patients were heavily pretreated with cytotoxic chemotherapy, the chances of obtaining a response or even long-term stabilization were slim; and (*c*) although we cannot document this other than by clinical observations, it is our impression that the DFMO-MGBG combination works best against those tumors that appear to grow slowly.

The use of DFMO is a novel approach to the treat-

TABLE 3.—Schedule I: patient characteristics*

Patient No.	Age (yrs)	Diagnosis	Prior treatment		KPS	Disease status	TTF (wks)
			Chemotherapy	Radiation therapy			
1	8	Medullo	X	X	80	D	8
2	7	Medullo	X	X	90	D	11
3	12	Medullo	X	X	90	D	6
4	5	Medullo		X	90	D	7
5	24	MOAA	X	X	90	D	8
6	40	HAA	X	X	90	S	68
7	24	HAA	X	X	60	D	11
8	51	GM	X	X	90	D	13
9†	64	GM	X	X	80	S	13
10†	51	GM	X	X	70	S	6
11	49	GM	X	X	80	D	10
12	19	BSTM	X	X	90	S	15

*KPS = Karnofsky performance score; Medullo = medulloblastoma; BSTM = unbiopsied brain stem glioma; D = immediate progression; and S = stable disease.
†Patients 9 and 10 were censored because they refused treatment due to gastrointestinal toxicity.

TABLE 4.—Schedule II: patient characteristics*

Patient No.	Age (yrs)	Diagnosis	Prior treatment: Chemotherapy	Prior treatment: Radiation therapy	KPS	Disease status	TTP (wks)
1	31	MOAA	X	X	70	R	36
2	14	MOAA	X	X	80	R	118
3†	28	MOAA	X	X	90	R	68
4	23	MOAA		X	80	S	27
5	42	MOAA	X	X	70	S	66
6†	37	MOAA		X	80	S	28
7	34	HAA	X	X	90	R	19
8†	29	HAA		X	90	S	25
9	44	HAA		X	80	S	8
10†	29	HAA	X	X	80	S	48
11†	36	HAA	X	X	90	S	21
12†	22	HAA	X	X	90	S	16
13	59	HAA	X	X	90	D	9
14	41	HAA	X	X	80	D	9
15	34	HAA	X	X	90	D	10
16	34	GA		X	90	S	27
17	18	EPEN	X	X	80	S	18
18	28	MMG (mixed cord)	X	X	60	D	8
19	29	OLIGO		X	90	D	10
20	35	GM	X	X	90	D	8
21	41	GM	X	X	90		8

*OLIGO = oligodendroglioma; R = responding patient; and EPEN = ependymoma.
†Censored patient.

ment of primary recurrent brain tumors. Although MGBG does cross the blood-brain barrier (34), DFMO does not cross as readily (23) and there is theoretical concern that this agent would not be appropriate to use. Levin et al (23,24) have reviewed this issue, which we briefly summarize here.

DFMO is quite stable in the body with > 95% being excreted intact in the urine (1,2,5). Furthermore, because it can be given by oral administration for periods of days to weeks, sustained plasma levels can be maintained for extended periods of time. In tumor-bearing rats, sustained plasma drug levels rapidly reached steady-state tumor levels and then diffused from the tumor into the surrounding brain to achieve brain/plasma ratios > 1 within days (23). Normal brain levels were not measured in this study, although CSF/plasma ratios were measured in rats and dogs undergoing long-term iv dosing. In those studies, CSF/plasma ratios of 0.10, 0.41, and 0.64 were obtained at 1, 7, and 14 (14-day extrapolated value) days, respectively, of a sustained iv dose and constant plasma DFMO drug level. Thus, it would be expected that DFMO exposures of 14 days would be adequate to reduce putrescine levels in tumor cells at a distance from leaky tumor vessels.

In conclusion, we believe that this study is important because it demonstrates, for the first time, substantial activity of a combination of two polyamine inhibitors, DFMO and MGBG, in the treatment of AGs. To further define the value of the combination of DFMO and MGBG in the treatment of AGs, we intend to evaluate DFMO-MGBG in a controlled clinical trial for patients with well-differentiated astrocytoma and oligodendroglioma; the arms of the study will be surgery alone versus surgery and DFMO-MGBG versus surgery and radiation therapy.

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Combination Chemotherapy and Hormonal Therapy in the Treatment of Malignant Melanoma[1,2]

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Twenty-three patients with metastatic melanoma were treated with combination therapy consisting of dacarbazine (220 mg/m²) and cisplatin (25 mg/m²) iv daily for 3 days every 3 weeks, carmustine (150 mg/m²) iv every 6 weeks, and tamoxifen (10 mg) orally twice daily. In 20 evaluable patients, there were no complete responses and ten partial responses. The median remission duration has not yet been reached but exceeds 7 months. Treatment was relatively well tolerated. However, six patients developed deep venous thrombosis, and four of these six suffered pulmonary emboli. Our data support a previous study and suggest that this combination warrants comparison with the active single components in a randomized prospective trial. [Cancer Treat Rep 71:465–469, 1987]

Treatment of patients with metastatic melanoma has been a particularly frustrating problem for the medical oncologist. There are few useful agents. Dacarbazine (DTIC) and the nitrosoureas [carmustine (BCNU), lomustine] have been the mainstays of therapy (1). No combination regimen containing one or both of these drugs has been demonstrated to be superior to the single agents in randomized trials.

Del Prete et al (2) have recently reported a clinical trial of combination therapy consisting of DTIC, BCNU, cisplatin (DDP), and tamoxifen. They treated 20 patients and obtained an overall response rate of 55% (11 of 20). Four patients obtained complete response (CR) including visceral disease, while seven patients achieved partial response (PR); reported toxicity was minimal. Based on these encouraging results, we conducted a trial using this regimen and here report our results in the first 23 patients treated.

MATERIALS AND METHODS

From December 1984 to June 1986, 23 consecutive patients were entered into this clinical trial. Entry requirements included histologically documented melanoma, a measurable lesion, Eastern Cooperative Oncology Group (ECOG) performance status of 0–2, and no antitumor therapy during the preceding 4 weeks. No effort was made to exclude patients on the basis of site of disease.

The treatment regimen consisted of: DDP (25 mg/m²) and DTIC (220 mg/m²) iv daily for 3 days every 3 weeks, BCNU (150 mg/m²) iv every 6 weeks, and tamoxifen (10 mg) orally twice daily. The DDP was preceded by iv hydration in addition to an antiemetic regimen consisting of dexamethasone, high-dose metoclopramide, and diphenhydramine hydrochloride. Patients had to complete 6 weeks (one cycle) of therapy before they were considered evaluable for response. After completion of the first cycle of therapy, the treatment interval was extended to 4 weeks for 18 of 20 patients due to delayed bone marrow recovery.

Responses were defined as follows: *CR*—the complete disappearance of all clinical evidence of active tumor, lasting 4 weeks; *PR*—a $\geq 50\%$ decrease in the sum of the perpendicular diameters of all measured lesions lasting at least 4 weeks, without progression of any other lesions (whenever possible a minimum of three lesions were measured); *progressive disease*—a $> 25\%$ increase in the sum of the perpendicular diameters of any measured lesions.

RESULTS

Twenty-three patients were entered into this study; three patients were deemed ineligible because they failed to complete one full cycle of treatment: one patient refused further therapy, another patient required surgery on a large fungating primary scalp lesion, and another exhibited rapidly progressive disease and died within 1 week of treatment. Pretreatment characteristics of evaluable patients are shown in table 1. Sites of

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TABLE 1.—Pretreatment characteristics

	No. of patients
Male/female	12/8
Age (yrs)	
Median: 47.5	
Range: 27–70	
Previous treatment	
None	10
Chemotherapy	2*
Radiotherapy	5*
Immunotherapy	4

*Patient 8 received both therapies.

TABLE 2.—Site of metastases in 20 patients

Site	No. of patients
Lung	12
Soft tissue	9
Sinus	2
Bone	2
Liver	2
Adrenal glands	1
CNS	1

metastatic disease prior to treatment are listed in table 2.

Twenty patients were evaluable for response and toxicity. There were no CRs and ten PRs. The clinical characteristics of patients who responded are listed in table 3. Magnitude of response is defined as the percent regression of all measured lesions (usually at least three) in addition to regression in unmeasured areas. Two responding patients (Nos. 2 and 9) developed progressive central nervous system (CNS) metastases while the original signal lesions were regressing. Patient 7 was having an excellent response when he decided to discontinue therapy after three cycles for personal reasons. He returned 5 months later with markedly progressive disease but once again refused further therapy and died 1 month later.

Of the nine patients currently alive, three are actively being treated and continue to show disease regression. Four have failed therapy and are undergoing other treatment. Two are being followed without treatment. Treatment was discontinued in patients 1 and 3 after four cycles of therapy, when tumor failed to regress further following an excellent initial response. Patient 1 developed vision changes 5 months after discontinuing treatment. Magnetic resonance imaging has revealed a metastatic lesion in the left optic nerve. Malignant cells were found in the cerebrospinal fluid; however, there is no evidence of regrowth of lung metastases. For Patient 3, it is now 10 months after treatment, without progression of disease. The only evidence remaining of prior disease is a 1-cm axillary lymph node that has not changed in size in > 1 year. Patient 8 discontinued treatment after three cycles due to poor tolerance, despite an excellent (95%) response. At present there is only a small (0.5-cm diameter) area of infiltrate on chest x-ray remaining as the lone indication of previous tumor, 15 months after therapy was stopped (figs 1A and 1B). Patient 10 presented with a solitary brain metastasis and massive bilateral adrenal metastases. He underwent surgical removal of the brain metastasis and subsequently received 3000 rads to the whole brain. He then began treatment as described with an excellent response after one cycle of therapy (figs 2A and 2B). He is presently still undergoing treatment.

Nine of the ten patients who responded did so after the first cycle. One patient exhibited disease stabilization after the first cycle and then disease regression following the next cycle.

The median duration of response is unknown at this time, as five of the ten responders continue in remission; it will exceed 7 months. Two patients have maintained their remission for > 1 year, despite discontinuing therapy after four cycles.

Toxicity

Toxicity encountered with this treatment is summarized in table 4. Hematologic toxicity was mild to moder-

TABLE 3.—Responder characteristics

Patient No.	Sex	Age (yrs)	ECOG performance status	Previous therapy	Metastatic sites	Duration of remission (mos)	Magnitude of response (%)
1	F	63	0	None	Lung	12	95
2	M	44	1	Immunotherapy	Lymph node, nasal mucosa	1.5	50
3	M	55	0	None	Lymph node, liver	16+	75
4	F	37	0	None	Lung	8+	50
5	F	64	0	None	Lung	10+	75
6	M	63	1	Radiotherapy	Liver	4	50
7	M	42	1	Radiotherapy	Sinus	6	75
8	F	70	0	Chemotherapy*	Lung	22+	95
9	M	34	2	None	Lymph node, bone	3	50
10	M	33	0	None	Brain, adrenal glands	4+	75

*Vinblastine and 5-fluorouracil.

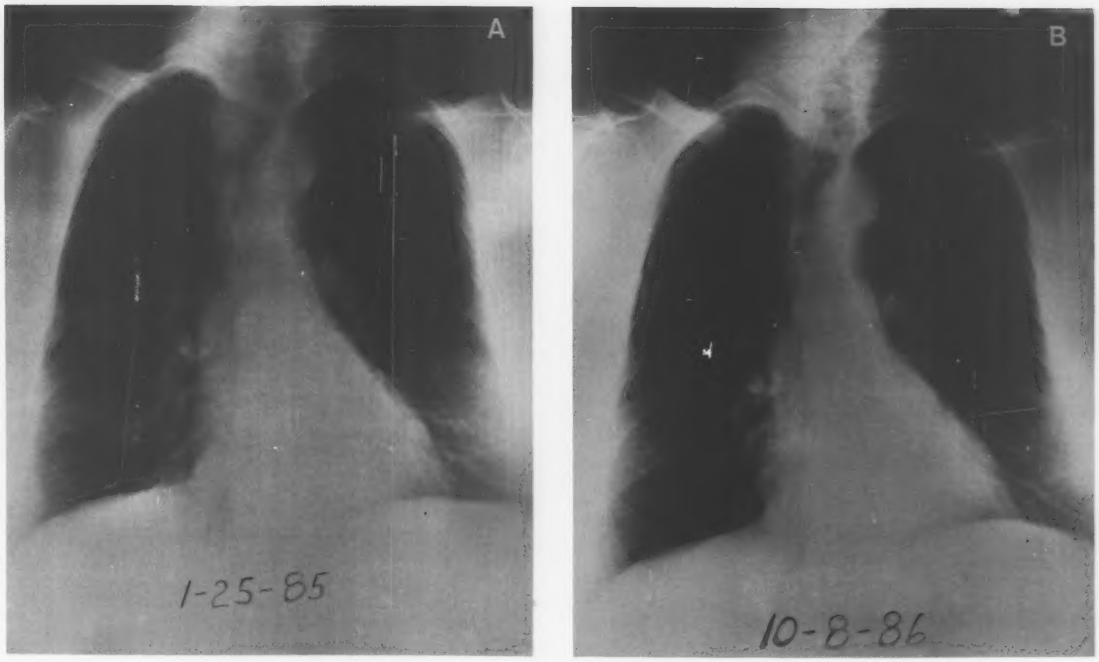

FIGURE 1.—Patient 8 prior to starting therapy (A) and 15 mos after completing 6 mos of therapy (B).

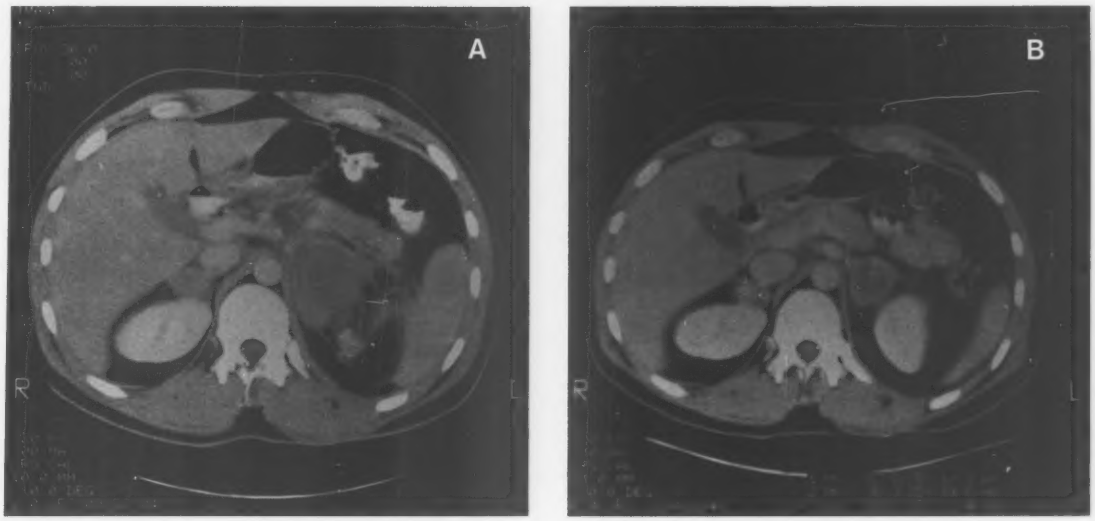

FIGURE 2.—Patient 10 at diagnosis, May 1986 (A), and after completing 1 cycle of therapy (B). Therapy was delayed until July 1986 for treatment of a solitary brain metastasis (surgery and whole-brain irradiation). Despite the slight difference in the size of these films, volumetric measurements of tumor mass revealed a 50% decrease.

TABLE 4.—Toxicity in 20 patients

Toxic effect	No. of patients (%)
Leukopenia (wbc count < 3000/mm^3)	13 (65)
Anemia (Hgb < 13 g/dl)	18 (90)
Thrombocytopenia (platelet count < 100,000/mm^3)	12 (60)
Renal (creatinine > 2.0 mg/dl)	3 (15)
Hypomagnesemia (Mg < 1.4 mEq/L)	16 (80)
Deep venous thrombosis/pulmonary embolism	6/4 (30)/(20)
Nausea/vomiting	16/12 (80)/(60)

ate in most cases. Although 13 patients developed leukopenia (wbc count ≤ 3000/mm^3), in only one patient did the wbc count fall below 1000/mm^3. This patient did not develop fever or require antibiotic therapy. Eighteen patients developed anemia while on therapy, and nine of these patients required at least one transfusion. Anemia typically occurred after the second cycle. Twelve patients experienced thrombocytopenia (< 100,000 cells/mm^3), but in only one patient was this clinically significant. The patient was admitted to the hospital with bleeding gums, purpura, and petechia and was found to have a platelet count of 6000/mm^3. Transfusions with platelets rapidly corrected this problem. Because of hematologic toxicity, in most patients treatment was repeated at 4-week intervals rather than every 3 weeks.

Renal toxicity was relatively mild, with two patients developing slight (< 2.5 mg/dl) elevations in serum creatinine. One patient had a moderate elevation (2.8 mg/dl) in serum creatinine, but 24-hour clearance revealed a significant reduction (11 ml/minute) in renal function. There was evidence of renal wasting of magnesium, as 16 of 20 patients developed hypomagnesemia requiring replacement therapy. This was symptomatic in three patients.

Gastrointestinal toxicity was limited to nausea and vomiting in most cases. Patients experienced the most difficulty on Day 1 of treatment. However, two patients experienced severe nausea and vomiting at 24–48 hours after discharge from the hospital. This was severe enough to prompt admission to their local hospitals with dehydration and electrolyte abnormalities. We suspect that this is due to an adynamic ileus likely induced by DDP. Administration of metoclopramide (10 mg orally, four times daily) for 5 days post-DDP has resolved this problem.

Six patients developed deep venous thrombosis (DVT) during therapy. This was confirmed by venogram in three patients. Three of these six patients had pulmonary emboli documented by ventilation perfusion scan. In a fourth patient a pulmonary embolus was diagnosed clinically on the bases of the sudden onset of respiratory distress and arterial hypoxemia, in addition to recent development of left lower extremity swelling.

DISCUSSION

The mainstay of treatment for metastatic melanoma has been DTIC or a nitrosourea. These single agents produce objective remissions in < 25% of the patients treated, and durable remissions are infrequent. Combination chemotherapy has done little to improve the response rate and has added significantly to toxicity (3–6). The report of Del Prete et al (2) suggested that the four-drug regimen of DTIC, BCNU, DDP, and tamoxifen may be an improvement over single-agent therapy. In general, our response rate of 50% (95% confidence interval of 27%–72%) confirms the 55% response rate (95% confidence interval of 32%–77%) previously reported. Although none of our patients experienced CR, responses approached 90% in several. Of particular importance is the improved response seen in patients with visceral disease. All but two responding patients in this study had documented regression of visceral metastases, with the lung being the most common site of response. It would not appear that this regimen is useful for treating CNS metastases, as two patients exhibited rapid progression of brain metastases despite continued response of peripheral disease.

Toxicity was similar in the two studies, except for our failure to observe pulmonary toxicity and the unexpected incidence of DVT. DVT is particularly disturbing because of the potentially life-threatening complication of pulmonary embolism. Four of our six patients with DVT developed this complication. Accepted risk factors for the development of DVT include a period of immobility, obesity, congestive heart failure, previous episode of DVT, medications, surgery, and cancer. Only two of the six patients had a period of immobility prior to the development of DVT. Although cancer is generally associated with an increased incidence of DVT, in reality this is seen most commonly in patients with mucin-producing adenocarcinomas. Melanoma is not associated with an increased risk of DVT.

Certain medications are accepted as risk factors for developing DVT. Tamoxifen was the only medication that these patients were taking which is associated with an increased risk of DVT. There are presently several reports (7–9) in the literature implicating tamoxifen in the development of DVT. The mechanism is unclear; however, depressed levels of anti-thrombin III activity have been found in patients treated with tamoxifen (10). There were no previous episodes of DVT or congestive heart failure in this patient population. None of the patients was obese or had undergone a recent surgical procedure.

Hormonal therapy has been disappointing in the treatment of melanoma. Most published response rates are < 10%. In light of this fact, one questions the inclusion of tamoxifen in this regimen. It is likely that it adds very little to the remission rate, yet in our study it may well be related to the increased rate of DVT. Accurate assessment of the contribution of tamoxifen to the antitumor activity of this regimen would require a randomized study of this combination with and without tamoxifen.

In summary, the two studies combined show an overall response rate of 53% (21 of 40 patients), with several individuals attaining remission durations > 1 year. These results are superior to the reported response rates with

the active single agents: DTIC—21% (293 of 1379 patients), $P < 0.01$; nitrosourea—14% (98 of 693), $P < 0.01$; and DDP—13% (ten of 78), $P < 0.01$ (11). The data justify performance of a randomized prospective comparison of the combination regimen with the active components.

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Hexamethylene Bisacetamide-Induced Cutaneous Vasculitis[1,2]

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Nonthrombocytopenic purpura developed in a patient receiving hexamethylene bisacetamide, a tumor-differentiating agent in vitro that is in phase I clinical trials. Histopathological examination revealed features of leukocytoclastic vasculitis with deposition of IgA and complement in the dermal vasculature. Leukocytoclastic vasculitis, an extremely rare complication of therapy with antineoplastic agents, is reported to alert others using this compound of this association. [Cancer Treat Rep 71:471-474, 1987]

Hexamethylene bisacetamide (HMBA) has been demonstrated to be the most effective of the polar-planar compounds at inducing differentiation in a wide variety of leukemic and nonleukemic cell lines (1,2). In a phase I and pharmacologic study of HMBA administered as a 5-day continuous infusion every 3 weeks, steady-state HMBA plasma concentrations were in the range of 1–2 mM, approaching the lower concentration limit demonstrated for in vitro differentiating effectiveness (3). Dose-limiting toxic effects were renal insufficiency, a hyperchloremic metabolic acidemia/acidosis, and central nervous system toxicity. Moderate myelosuppression, mucositis, nausea, and vomiting were also observed. No dermatologic, vasculitic, or allergic complications were noted. Since steady-state plasma concentrations were lower than desired to produce differentiation, a second trial utilized a 10-day continuous infusion schedule in which a case of cutaneous leukocytoclastic vasculitis was noted. Leukocytoclastic vasculitis is an extremely rare complication of antineoplastic therapy and it has not been previously described with administration of HMBA.

CASE REPORT

A 65-year-old female was well until May 1984, at which time she developed upper gastrointestinal bleeding and syncope. An evaluation led to the diagnosis and resection of a leiomyosarcoma of the jejunum. She remained asymptomatic until June 1985, when she developed right upper quadrant pain. A computerized tomographic scan revealed multiple filling defects in the liver and a liver biopsy showed metastatic leiomyosarcoma. From August 1985 to January 1986, she was treated with doxorubicin and later with trimetrexate, but these drugs were discontinued due to progressive tumor growth.

The patient was subsequently treated with HMBA administered at a dose of 15.8 g/m^2/day as a continuous infusion daily × 10. No toxicity was noted during the infusion of her first course; however, a macular erythematous periorbital and malar rash appeared on Day 12 and progressively worsened until Day 15 (fig 1A). She had no other complaints. Physical examination, cbc, urinalysis, electrolytes, and renal and liver function tests were unremarkable. Antinuclear antigen (ANA) using HEp 2 cells as a substrate was diluted at a titer of 1:40 with a homogeneous pattern. The rash resolved totally by Day 22.

FIGURE 1A.—Periorbital and malar rash.

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FIGURE 1B.—Palpable purpura involving both lower extremities.

On Day 3 during the infusion of her second course of HMBA, multiple 1-2-mm papular erythematous lesions developed on both ankles which soon coalesced and were identified as palpable purpura. She exhibited no other signs or symptoms of vasculitis. Medications included hydromorphone and docusate sodium. Physical examination, cbc, electrolytes, and renal and liver function tests remained unremarkable. ANA was persistently positive at a 1:80 titer and a homogeneous pattern. Histopathological examination of a skin biopsy obtained on Day 3 revealed focal epidermal necrosis and an extensive inflammatory cell infiltrate in the dermis which consisted of mononuclear cells, lymphocytes, eosinophils, and scattered and degenerated neutrophils (fig 2A). Endothelial swelling, dermal hemorrhage, and intravascular and perivascular fibrin deposition were also present. Direct immunofluorescence studies revealed granular deposits of IgA and C3 in the dermal vasculature (fig 2B). These histopathological findings were felt to be consistent with a drug-induced leukocytoclastic vasculitis mediated by IgA immune complexes. However, circulating immune complexes were not detected by a Clq binding assay. C3 and C4 were 111 mg/dl (normal range, 83-177) and 17 mg/dl (normal range,

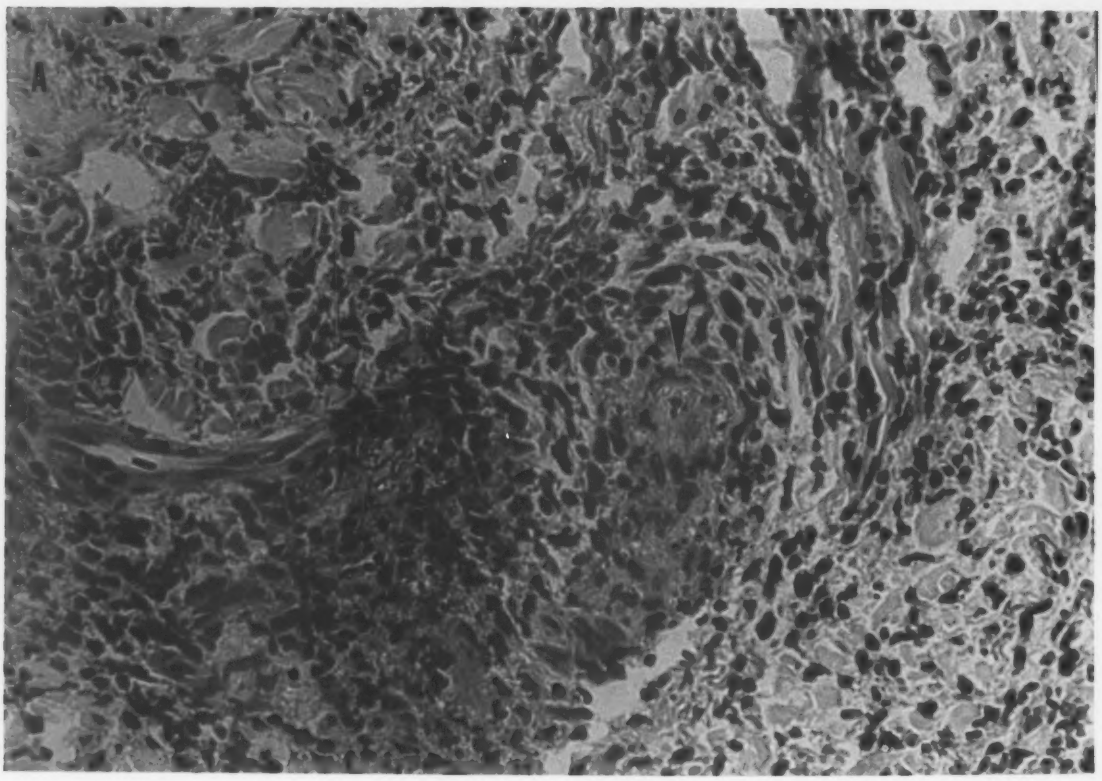

FIGURE 2A.—Photomicrograph shows hemorrhage and fibrinoid necrosis with obliteration of blood vessels (arrow) and inflammatory infiltrate consisting of lymphocytes, eosinophils, and neutrophils with leukocytoclasis in the reticular dermis (× 914).

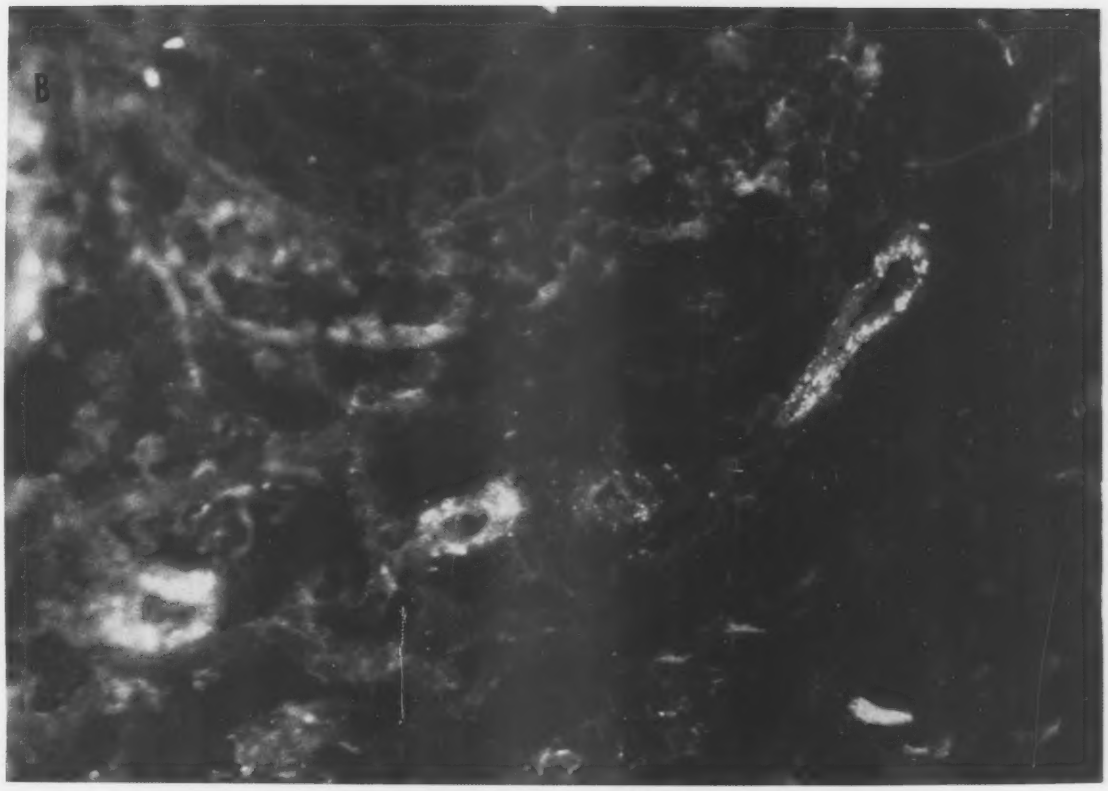

FIGURE 2B.—Direct immunofluorescence reveals granular deposition of IgA in the dermal vasculature (× 914).

15–45), respectively. Although the rash remained confined to her ankles for several days, the purpuric lesions subsequently became confluent and progressed to her midthighs by Day 8 (fig 1B). She also redeveloped her malar rash. HMBA was discontinued on Day 8 prior to completion of the 10-day infusion, and prednisone at 20 mg twice a day was begun. All skin lesions resolved completely by Day 22.

DISCUSSION

The patient described developed palpable purpura with histologic features of leukocytoclastic vasculitis during her second course of treatment with HMBA. Manifestations of vasculitis were limited to the skin. A macular facial rash appeared following her initial course of HMBA and reappeared on rechallenge. This rash was felt to be a separate clinical entity; its nonpurpuric and nonurticarial features, different time course, and occurrence on a nondependent region were uncharacteristic of leukocytoclastic vasculitis and more characteristic of a less severe drug eruption.

No literature exists that implicates classic cytotoxic agents as causing leukocytoclastic vasculitis. However, cutaneous necrotizing vasculitis has been recognized as a complication of therapy with the biologic response modifier alpha-interferon (4). Medications that are more commonly incriminated include penicillins, sulfonamides, thiazides, procainamide, quinidine, aspirin, phenacetin, and allopurinol (5,6). Other precipitating conditions include infections with hepatitis B virus and group A hemolytic streptococci, autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus, and exposure to such chemicals as pesticides and petroleum products (5–8). Leukocytoclastic vasculitis has also been noted to occur in association with several malignancies including Hodgkin's and non-Hodgkin's lymphomas (9). The patient described had none of these risks.

Current evidence suggests that leukocytoclastic vasculitis results from the deposition of immune complexes in the vessel walls with the drug or metabolites acting as antigenic stimuli (8,10). Although the patient described did not develop skin lesions characteristic of leukocytoclastic vasculitis during her first course of HMBA, it is possible that sensitization or priming of her immune system occurred at that time. Reintroduction of the causative antigen during her second course potentially stimulated a further increase in antibody

production resulting in clinically significant immune complex formation. Although the deposition of IgA in the dermal vasculature is more characteristic of Henoch-Schönlein purpura (11), IgA and C3 deposition may also occur in many other varieties of vasculitis including drug-induced forms (7,12,13). Immunoglobulin and C3 have even been detected in the dermal blood vessels of biopsies of clinically normal skin of patients with active necrotizing vasculitis and palpable purpura, which has suggested that immunoglobulin and complement deposition occurs prior to chemotaxis and the development of the inflammatory purpuric lesions (12).

The findings of normal levels of the serum complements C3 and C4 and failure to demonstrate circulating immune complexes are not uncharacteristic of drug-induced leukocytoclastic vasculitis. Hypocomplementemia is actually an uncommon manifestation of drug-induced dermal vasculitides and is more characteristic of vasculitides resulting from the collagen-vascular diseases, cryoglobulinema, or the separate clinical entity hypocomplementemic vasculitis (6). Failure to demonstrate circulating immune complexes may be due to the low sensitivity of the assay used (Clq); the different sizes, composition, and clearance rates of the immune complexes; or the time of serum sampling relative to the onset of symptoms (7). Although our patient's skin lesions resolved following the administration of steroids, there is no good evidence that any form of therapy, except the withdrawal of the suspected etiologic agent or antigenic stumuli, alters the course of this complication.

In conclusion, leukocytoclastic vasculitis and drug eruptions may occur as a result of therapy with HMBA. Because of the potential for increased use of HMBA in the treatment of malignant and premalignant disorders, physicians should be aware of the potential for HMBA-induced cutaneous vasculitis so that more serious systemic vasculitic complications may be avoided.

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Third-Line Chemotherapy for Resistant Hodgkin's Disease With Lomustine, Etoposide, and Methotrexate[1,2]

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Thirty-two patients with recurrent Hodgkin's disease have been treated with an oral regimen employing lomustine (CCNU, 100 mg/m^2 orally on Day 1); etoposide (VP-16, 100 mg/m^2 orally on Days 1-3 and 21-23); and methotrexate (30 mg/m^2 orally on Days 1, 8, 21, and 28). The regimen was repeated every 6 weeks. Most patients had been treated with MOPP (mechlorethamine, vincristine, procarbazine, and prednisone) and ABVD (doxorubicin, bleomycin, vinblastine, and dacarbazine); 20 had had prior irradiation. Lymph node was the predominant site of disease and the majority of patients had B symptoms. Four patients achieved complete response (13%), with a median duration of 33+ months, and 11 achieved partial response (34%), with a median duration of 5 months, for an overall response rate of 47%. The major toxic effect was severe myelosuppression, which occurred in six patients; there were no treatment-related deaths. This oral regimen was easy to administer in heavily pretreated patients with poor venous access and had minimal toxicity. [Cancer Treat Rep 71:475-478, 1987]

The treatment of relapsed Hodgkin's disease following radiotherapy and combination chemotherapy remains problematic. The major obstacle to complete remission (CR) is the selection and overgrowth of a drug-resistant, neoplastic cell population (1). Patients who have failed MOPP (mechlorethamine, vincristine, procarbazine, and prednisone) chemotherapy and radiotherapy have been treated with a variety of second-line chemotherapy regimens. These include reinduction MOPP (2), ABVD (doxorubicin, bleomycin, vinblastine, and dacarbazine) (3), BCAVe [bleomycin, lomustine (CCNU), doxorubicin, and vinblastine] (4,5), ABDIC (doxorubicin, bleomycin, dacarbazine, CCNU, and prednisone) (6), SCAB (streptozocin, CCNU, doxorubicin, and bleomycin) (7), and others (8). The success rate of these second-line regimens is relatively poor, achieving 5-year actuarial survival rates of only 20%-25% (1,5).

Patients who continue to have refractory Hodgkin's disease have been treated with either third-line regimens (9-11), bone marrow transplantation (12-14), or palliative single-agent therapy (15). The question of whether long-term remission can be achieved with any of these modalities will require further follow-up and evaluation. A new all-oral third-line regimen for the treatment of resistant Hodgkin's disease is presented in this report. It is easily administered, well-tolerated, and shows comparable activity to other third-line regimens (10).

MATERIALS AND METHODS

Between April 1982 and June 1986, 32 patients with recurrent Hodgkin's disease were entered in this study. Eligibility criteria included wbc count > 3000/mm^3, platelet count > 100,000/mm^3, Karnofsky performance status > 60%, and resistance to MOPP and then later to ABVD or BCAVe, or the alternating regimen of MOPP/ABVD, as indicated by either progressive disease during therapy or relapsed disease after an initial CR. All eligible patients were entered.

The CEM regimen consisted of CCNU (100 mg/m^2 orally on Day 1), etoposide (VP-16; 100 mg/m^2 orally on

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Days 1-3 and 21-23), and methotrexate (30 mg/m^2 orally on Days 1, 8, 21, and 28). Treatment was repeated every 6 weeks. CR was defined as the complete disappearance of all measurable disease for a minimum of 6 weeks; and partial response (PR) was defined as a $\geq$ 50% regression of measurable disease (as measured by the product of perpendicular diameters of measurable disease sites), with no new site of malignancy developing. After CR was achieved, two additional cycles of chemotherapy were administered, with a minimum of six cycles given. Patients had blood cell counts and liver function tests performed every 3 weeks. Toxicity was graded according to Northern California Oncology Group criteria. Confidence intervals were calculated by the method of Fleiss (16).

RESULTS

Thirty-two patients with refractory Hodgkin's disease met the eligibility criteria and all were evaluable for response and toxicity. There were 22 males and ten females, with a median age of 33 years (range, 16-52). Nodular sclerosing histology was the most prevalent subtype (25 patients), followed by mixed cellularity (six patients) and unclassified (one patient). There were 16 patients who had progressive disease while receiving either sequential or alternating MOPP/ABVD or MOPP/BCAVe chemotherapy. The other 16 patients had recurrent disease ranging from 3 months to 5 years after CR. The majority of patients had B symptoms and a Karnofsky performance status > 80% (table 1). Thirteen patients had only lymph node involvement at the time of recurrent disease.

There were four CRs lasting 7+, 10, 41+, and 50+ months. One of these patients relapsed at 10 months and is currently receiving palliative single-agent chemotherapy. All four CRs were achieved in patients who had lymph node disease only and occurred after three to four cycles of chemotherapy were administered. One CR occurred in a patient who had progressive disease on MOPP/ABVD chemotherapy. The other three CRs occurred in patients who had recurrent disease after being in remission for 6, 24, and 44 months. Eleven patients achieved PR (34%), with a median duration of 5 months (range, 3-8). The overall response rate was 47% (90% confidence interval, 32%-63%). The median overall survival from the time of last relapse and initiation of the CEM regimen was 6 months.

Toxicity

Myelosuppression was the major toxic effect. Six patients had a platelet count nadir < 50,000/mm^3, and two required platelet transfusions. Three patients developed severe anemia (Hct < 25 mg/100 ml). A wbc count nadir < 1000/mm^3 occurred in two patients, both of whom were hospitalized for fevers. Mild to moderate nausea occurred in eight patients. Seven patients developed alopecia. Uncommon side effects included one episode each of lightheadedness, weakness, myalgias, diarrhea, and mucositis.

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total	32
Stage of disease at 1st presentation	
I	1
II	11
III	11
IV	9
B symptoms	23
Karnofsky performance status (%)	
90-100	12
80-89	11
70-79	3
60-69	6
Predominant sites of recurrent disease	
Lymph nodes	22
Lung	8
Bone	5
Bone marrow	5
Spleen, gastric	3
Liver	2
Prior therapy	
MOPP/ABVD (sequential/alternating)*	18/12
MOPP/BCAVe (sequential)	2
Radiotherapy	20
Status at time of recurrence	
Progressive disease	16
Recurrence of therapy after CR	16

*Sequential: MOPP regimen was completed and patient then received the ABVD regimen; alternating: MOPP regimen was alternated with ABVD regimen every other mo.

DISCUSSION

The CEM regimen was designed as an outpatient, all-oral regimen which could be easily administered and tolerated by heavily pretreated patients with resistant Hodgkin's disease. VP-16 was administered orally; pharmacokinetic data show the bioavailability to be approximately 50% when given in this manner as compared to iv administration (16-19,25). Therefore, twice the iv dose was given, based on myelosuppression. All three agents have demonstrated activity in the treatment of advanced, recurrent Hodgkin's disease. CCNU has been used at doses ranging from 100 to 130 mg/m^2, achieving response rates of 53%-60% and a median duration of response of 4.5 months (21). VP-16 has been administered at doses ranging from 40 to 100 mg/m^2, achieving response rates of 14%-23% and a median duration of response of 3.5 months (22,23). Methotrexate, at a dose of 0.15 mg/kg/day, achieved a response rate of 50% (four patients) and a duration of response of 8.5 months (24). It was originally incorporated into two quadruple drug combinations by the National Cancer Institute (26,27), but has the least well-established activity against Hodgkin's disease (28). Although the response rate of this reg-

TABLE 2.—Third-line chemotherapy regimens

Investigators	Ref No.	Regimen	Total No. of patients	CR (%)	Median duration of CR (mos)	CR + PR (%)
Bonadonna et al	9	CEP	58	40	>15	54
Straus et al	10	CAD	15	13	4	46
Tannir et al	11	MIME	46	24	>17	61
Current study		CEM	32	13	>27	47

imen is no better than CCNU, the CR rate is higher and the duration of response superior to that achieved by single-agent therapy.

The CEM regimen achieved an overall response rate of 47%, which is comparable to other reported series of third-line regimens (table 2).

The CEP regimen (CCNU, VP-16, and prednimustine) was one of the first third-line chemotherapy regimens tested (9). The drug doses used in the CEP regimen were as follows: CCNU, 80 mg/m^2 orally on Day 1; VP-16, 100 mg/m^2 orally on Days 1–5; and prednimustine, 60 mg/m^2 orally on Days 1–5. In 58 treated patients, a 40% CR rate and a 14% PR rate were achieved. Median duration of relapse-free survival was >15 months, and overall survival of patients with CR was >24 months. However, severe thrombocytopenia occurred in eight patients who had previously received extensive irradiation. The overall response rate with the CEP regimen (54%) is similar to the response rate in this study (47%). However, the number of CRs was greater with the CEP regimen. This may be related to the 5-day treatment with VP-16.

With another third-line combination, CAD (CCNU, melphalan, and vindesine), a response rate of 46% was achieved in 15 patients (10). Of 46 patients treated with MIME (mitoguazone, ifosfamide, methotrexate, and VP-16), 60% achieved response (11). With the MIME regimen, the absence of extranodal involvement was the only statistically significant factor predicting for CR (66% vs 16%) (1). Toxicity with both the CAD and MIME regimens was significant. Neutropenia, sepsis, and infection were seen, and with the MIME regimen there was one fatal hemorrhagic episode.

The successful use of high-dose chemotherapy with autologous bone marrow transplantation in this clinical setting has been reported (12–14). In the largest series, 30 patients with relapsed Hodgkin's disease were treated with cyclophosphamide, carmustine, and VP-16 with autologous bone marrow transplantation (12). Fifteen patients achieved CR, with a median duration of 9 months (range, 5–44+). Five CRs occurred in 14 patients with lymph node disease only (36%), and ten CRs occurred in 16 patients (63%) with both lymph node and extranodal disease.

The CR rate of the CEM regimen for patients relapsing with lymph node disease only is similar to that attained with high-dose chemotherapy and autologous bone marrow transplantation. With the CEM regimen, four CRs occurred in 13 patients; with the high-dose chemotherapy with autologous bone marrow transplantation program, five CRs occurred in 14 patients. Therefore, relapsed patients with lymph node disease only could be treated with CEM chemotherapy and be spared the toxic effects and expense of high-dose chemotherapy with autologous bone marrow transplantation with the same anticipated outcome. Patients relapsing in extranodal sites may achieve better results with a transplant. It is realized that the number of patients in both the transplant series as well as the current study is small, and that accession of more patients will be needed before firm recommendations can be made. Additionally, these drugs can be incorporated into hybrid second-line regimens, similar to the ABVD/CEP program of Bonadonna et al (20).

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Effect of Amsacrine on Ara-CTP Cellular Pharmacology in Human Leukemia Cells During High-Dose Cytarabine Therapy[1,2]

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The combination of high-dose cytarabine (ara-C) and amsacrine (*m*-AMSA) is effective treatment for relapsed adult acute leukemia. Studies were performed to determine if *m*-AMSA affected the pharmacokinetics of the active triphosphate ara-CTP in HL-60 and K562 cells in culture. No significant differences were observed in accumulation, rate of elimination, or total intracellular exposure to ara-CTP in cultures treated with 100 μM ara-C alone or in combination with 1 μM *m*-AMSA. In clinical investigations, the accumulation and retention of ara-CTP in circulating leukemic cells were studied in five patients after two serial doses of ara-C (3 g/m² infused over 2 hours) and in six additional patients in whom the second dose of ara-C was accompanied by an infusion of *m*-AMSA (30 mg/m² infused over 1 hour). While substantial differences were observed in the cellular pharmacokinetics of ara-CTP among patients, the rate of ara-CTP elimination and the total intracellular exposure to ara-CTP in individuals were remarkably similar after each ara-C infusion. Infusion of *m*-AMSA with the second dose of ara-C did not significantly affect the cellular pharmacokinetics of ara-CTP. These studies demonstrate the feasibility and utility of conducting investigations of the cellular pharmacology of drug-drug interactions in human leukemic cells during therapy. [Cancer Treat Rep 71:479–483, 1987]

The pyrimidine nucleoside analog cytarabine (ara-C) is the most effective drug for the treatment of adult acute myelogenous leukemia (1). The cytotoxic activity of ara-C is dependent on its intracellular conversion to the active 5′-triphosphate ara-CTP (2). Ara-CTP competes with dCTP in the inhibition of DNA polymerase activity (3,4), but its incorporation into DNA indicates that it also serves as a substrate in the polymerization reaction (5,6). These actions are associated with the cytotoxicity of the drug (7). However, ara-CTP also participates as an analog in the intermediary metabolism of phospholipids and in glycoprotein synthesis (8,9) and at high concentrations may inhibit RNA polymerase activity (10).

Serial sampling of peripheral blood during and following ara-C infusions has displayed a pharmacokinetic picture of ara-CTP metabolism in leukemic cells (11–13). These studies have permitted comparison of the variability of these parameters within the same patient and have enabled a search for correlations with clinical response. Substantial heterogeneity was seen among patients with respect to the ability to accumulate and retain ara-CTP. Some characteristics of the cellular pharmacokinetics of ara-CTP in circulating blasts of patients with relapsed acute leukemia were strongly correlated with clinical response to high-dose ara-C therapy. While the peak ara-CTP values were not significantly different between patients who achieved complete remission and those who failed to respond to treatment, significant relationships were found between response and the rate of ara-CTP elimination, the trough ara-CTP concentration, and the area under the ara-CTP accumulation and retention curve. However, because ara-C is usually administered as one drug in a combination with other agents, it is of importance to consider the effect that other drugs might have on the cellular metabolism of ara-CTP.

Preclinical investigations have demonstrated that when used in combination with ara-C, drugs such as hydroxyurea (14,15) and 3-deazauridine (16,17) may enhance the total intracellular exposure to ara-CTP. We have presented evidence that the 3-deazauridine effect may

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also be seen during therapy for patients with acute leukemia treated with this combination (18). On the other hand, methotrexate (19) and epipodophyllotoxins (20) may limit ara-CTP accumulation.

Another drug with significant antileukemic activity, amsacrine (*m*-AMSA) (21), is currently undergoing clinical trials in combination with ara-C. Available evidence suggests that the antileukemic activity of the combination may be greater than the sum of that evoked by each drug alone (22–24). To determine whether possible synergistic action might result from an effect on ara-CTP pharmacokinetics in leukemic cells, the present investigation was designed to evaluate the effect of *m*-AMSA on ara-CTP metabolism in human leukemic cell lines and then in leukemic blasts during therapy of adult acute leukemia with high-dose ara-C and *m*-AMSA.

PATIENTS AND METHODS

Patients

Adults with acute leukemia were treated with either of two therapeutic protocols. In the first protocol, patients with acute leukemia in relapse received ara-C alone in a phase II high-dose regimen (12). This consisted of four to 12 doses of 3 g/m^2 of ara-C infused over a 2-hour duration at 12-hour intervals. A second, two-arm combination chemotherapy protocol was used for patients with previously untreated acute leukemia. Patients received either six doses of high-dose ara-C at 12-hour intervals or two doses of 3 g/m^2 infused over 2 hours at 12-hour intervals followed by a continuous infusion of ara-C (70 mg/m^2) for 7 days. Each treatment arm required the administration of *m*-AMSA (30 mg/m^2) infused iv over 1 hour simultaneously with the second 2-hour infusion of ara-C. The design of these protocols made it possible to study the cellular ara-CTP pharmacokinetics after serial doses of ara-C alone (Protocol 1) and after consecutive doses of ara-C where the second dose was accompanied by a simultaneous infusion of *m*-AMSA (Protocol 2). The clinical status of all patients evaluated in this study is shown in table 1.

Cellular Pharmacology Studies

Informed consent for these investigations was obtained from all patients. Samples of peripheral blood (10 ml) were obtained at 1–2-hour intervals for 24 hours starting with the first dose of ara-C. Mononuclear cells were isolated by Ficoll-Hypaque density gradient centrifugation. Cells from each sample were counted and their mean cell volume was determined using an electronic particle counter that was equipped with a cell-sizing apparatus (Coulter Electronics, Inc, Hialeah, FL). Cellular nucleotides were extracted with $HClO_4$ and separated using anion-exchange high-pressure liquid chromatography, as described previously (11). Quantitation of absorbance at 280 nm was determined by electronic integration and referenced to preprogrammed response factors. Determination of the absolute quantity of ara-CTP contained in the $HClO_4$-soluble material extracted from a given number of cells of known mean cell volume permitted the expression of cellular contents of ara-CTP in terms of concentration. The total intracellular exposure to ara-CTP was calculated as the area under the curve (AUC) of ara-CTP accumulation and elimination (25,26). In this calculation the area under the linear accumulation phase was summed with that under the monoexponential elimination phase. The ara-CTP AUC values were expressed in units of μM-hr. The ara-CTP μM-hr value calculated to remain in the cells after the first dose of ara-C was subtracted from the ara-CTP AUC value determined after the second dose of ara-C to correct for this spillover effect.

TABLE 1.—Patient characteristics*

Patient No.	Diagnosis†	Wbc count ($\times 10^{-3}$ cells/μl)	Leukemic cells (%)	Prior therapy with ara-C
		Protocol 1		
1	AML	50.0	95	Refractory
2	ALL	29.7	85	Refractory
3	ALL	61.1	82	Refractory
4	AML	92.6	100	Relapsed off ara-C
5	ALL	46.3	100	No prior ara-C
		Protocol 2		
6	ALL	8.5	92	No prior ara-C
7	ALL	70.3	95	No prior ara-C
8	AMML	67.8	92	No prior ara-C
9	ALL	131.0	99	No prior ara-C
10	AML	34.7	94	No prior ara-C
11	AMML	73.0	87	No prior ara-C

*Wbc count in the peripheral blood immediately before therapy and the % of leukemic cells contained in the mononuclear fraction of the differential are given. Prior ara-C therapy refers to treatment with conventional doses of ara-C by continuous infusion. Specifics of each treatment protocol are defined in the text.

†AML = acute myelogenous leukemia; ALL = acute lymphocytic leukemia; and AMML = acute myelomonocytic leukemia.

Cell Culture Studies

Human promyelocytic cells (HL-60) and a line derived from a patient with chronic myelogenous leukemia (K562) were maintained in exponential growth in RPMI-1640 (GIBCO, Grand Island, NY) containing 10% fetal calf serum (KC Biologicals, Lenexa, KS). To determine the effect of *m*-AMSA on the accumulation and retention of ara-CTP, cultures of each cell line were incubated with 100 μM ara-C in the presence or absence of 1.0 μM *m*-AMSA. These concentrations were representative of those achieved in plasma during high-dose ara-C infusion (26) and *m*-AMSA administration (27). After 2 hours, cells were washed twice and resuspended in drug-free medium. Duplicate portions of each culture containing 5×10^6 cells were removed at 0.5-hour intervals, nucleotide extracts were prepared, and ara-CTP was separated from cellular nucleotides as described above. Ara-CTP was usually quantitated by monitoring UV absorbance at 280 nm. In one experiment with HL-60 cells that utilized [^{3}H]ara-C (specific activity, 3.91×10^7 dpm/μmol), similar results were obtained when ara-CTP was quantitated using radioactivity determinations and absorbance measurements. The values presented are the average of two determinations in one of the two experiments that were performed.

The pharmacokinetics of ara-CTP in leukemic cells were compared using Student's t test.

RESULTS

The effect of *m*-AMSA on ara-CTP retention by human leukemic cell lines was studied to provide a background for the clinical investigations. Figure 1 shows the levels of ara-CTP accumulated by HL-60 cells (fig 1A) and K562 cells (fig 1B) after a 2-hour incubation with 100 μM ara-C in the presence or absence of *m*-AMSA. No discernible differences were seen in the accumulation of ara-CTP by either cell line during the 2-hour incubation (shown as zero time) as a result of the addition of *m*-AMSA. Similarly, the rates of elimination of ara-CTP in each cell line appeared to be unaffected by the incubation with *m*-AMSA. The half-life of elimination of ara-CTP in HL-60 cells was 1.0 hour in each culture, whereas the ara-CTP elimination in K562 cells was 1.3 hours in cells treated with *m*-AMSA and 1.4 hours in those cultures incubated with ara-C alone. The area under the ara-CTP accumulation and elimination curve is a measure of the total intracellular exposure to the active metabolite (25). The AUC value for HL-60 cells incubated with ara-C alone was 105 μM-hr, whereas that for cells incubated with ara-C and *m*-AMSA was 113 μM-hr. The corresponding values in K562 cells were 99 and 116 μM-hr, respectively.

The design of the two treatment protocols permitted us to investigate whether *m*-AMSA administered simultaneously with ara-C would affect the cellular metabolism of the active metabolite ara-CTP. To provide a frame of reference for this investigation, we first determined the variability in the cellular pharmacokinetics of ara-CTP in the circulating blasts of patients who received treatment with two doses of ara-C alone. Whereas there were substantial differences in the rates of ara-CTP elimination seen among patients (table 2), there was no significant change in the ara-CTP elimination rates determined after serial ara-C infusions in the cells of each patient. Similar serial measurements in the circulating leukemic cells of patients who received

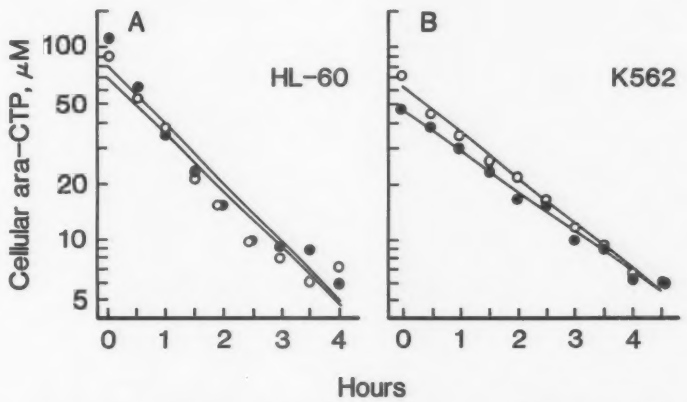

FIGURE 1.—Effect of *m*-AMSA on the accumulation and retention of ara-CTP by HL-60 cells (A) and K562 cells (B). Cells were incubated with 100 μM ara-C in the absence (○) or presence (●) of 1 μM *m*-AMSA for 2 hrs and washed in drug-free medium, and portions of each culture were analyzed for cellular ara-CTP concentrations at the indicated times. Each point represents the average of 2 determinations after cells were washed in drug-free medium.

TABLE 2.—Effect of *m*-AMSA on the ara-CTP elimination rates from leukemic cells*

Patient No.	Ara-CTP elimination half-life (hrs) for— Dose 1	Dose 2	% Change
	Ara-C†		
1	3.6	4.3	19
2	8.5	8.2	−4
3	2.0	2.4	20
4	5.8	6.4	10
5	9.6	6.4	−33
	Ara-C + m-*AMSA*‡		
6	2.7	3.0	11
7	3.3	3.5	6
8	3.4	2.5	−26
9	6.1	5.5	−10
10	3.5	3.5	0
11	3.7	3.0	−19

*Separate groups of patients with acute leukemia were treated with either intermittent doses of ara-C (3 g/m^2 infused over 2 hrs) at 12-hr intervals alone or with this regimen and an infusion of *m*-AMSA (30 mg/m^2) infused over 1 hr simultaneously with the second dose of ara-C. Rates of ara-CTP elimination, given as half-life (hrs), in circulating leukemic cells were determined after each dose of ara-C as described in the Materials and Methods section. % change reflects the ara-CTP elimination rate after the second ara-C infusion relative to the elimination rate observed after the first infusion.

†Median % change = 10%.

‡Median % change = −5%.

m-AMSA with the second dose of ara-C indicated that *m*-AMSA has little effect on cellular elimination of ara-CTP.

Although determinations of intracellular ara-CTP AUC values in the cells of patients who received only ara-C revealed both increases and decreases, the AUC values after the second dose of ara-C differed by > 16% in only one patient (table 3). The median change in the ara-CTP AUC values between the two doses of ara-C was a decrease of 5%. When the ara-CTP AUC was determined after administration of ara-C alone and then with *m*-AMSA, the median decrease in intracellular ara-CTP AUC was 17% (table 3). Four of the six patients studied in this protocol showed decreased ara-CTP AUC values during the simultaneous infusion of *m*-AMSA. The changes in the pharmacokinetics of ara-CTP in leukemic cells from patients treated in the two protocols were not significantly different, although the decreases in ara-CTP AUC that accompanied infusion of *m*-AMSA tended to be greater than those recorded after consecutive doses of ara-C.

DISCUSSION

The results indicate that neither the rate of ara-CTP elimination nor the ara-CTP AUC in circulating leukemic cells was significantly different in the cells of individual patients after serial doses of ara-C. This relative constancy was the basis for using intracellular ara-CTP pharmacokinetic parameters to direct the intervals between doses and the dose levels of ara-C on separate protocols (11). Given the complexity associated with the repeated sampling required to construct the pharmacokinetic profiles in the cells of each patient and the likely possibility of ongoing cellular damage, the level of variation seen in the cells of the patients who received ara-C alone was considered to be remarkably small.

Simultaneous infusion of *m*-AMSA with the second dose of ara-C had little effect on the cellular pharmacokinetics of ara-CTP. The intracellular exposure to ara-CTP decreased by a median value of only 17% (table 3), and the rate of ara-CTP elimination was slightly increased (table 2). Similar results were seen in human leukemic cell lines treated with these drug combinations (fig 1). The variation of the ara-CTP pharmacokinetic parameters in patients who received two doses of ara-C alone was sufficient to rule out finding significant effects of *m*-AMSA on the cellular metabolism of ara-CTP.

Differences in the ara-CTP pharmacokinetics are evident after a second dose of ara-C alone. This may reflect ara-CTP-induced damage to a specific cohort of leukemic cells in the population that is immediately susceptible to its action. With regard to the combination, clinical evidence that ara-C and *m*-AMSA together are more effective in inducing antileukemic response than the sum of activities of each agent alone suggests that cells exposed to the combination sustain greater damage than if they were treated only with a single drug (22–24). We have recently provided presumptive biochemical evidence for this likelihood by demonstrating DNA strand breaks in leukemic cells during therapy with *m*-AMSA (28). This suggests that pharmacokinetic determinations at later time points might be more likely to reveal *m*-AMSA-induced alterations in the cellular metabolism

TABLE 3.—Effect of *m*-AMSA on the area under the ara-CTP accumulation and elimination curve (AUC) in leukemic blasts*

Patient No.	AUC (μM-hr) for— Dose 1	Dose 2	% change
	Ara-C†		
1	1670	1940	16
2	5240	3400	−35
3	580	600	3
4	6370	6040	−5
5	6120	5450	−11
	Ara-C + m-*AMSA*‡		
6	1180	1690	43
7	3920	3520	−10
8	1340	1470	10
9	5300	4080	−23
10	5870	4470	−24
11	1540	1100	−29

*Ara-CTP AUC, expressed as μM-hr, was calculated for the patients presented in table 1. % change in the ara-CTP AUC value for the second dose of ara-C relative to that produced by the first ara-C dose was calculated as described in the Materials and Methods section.

†Median % change = −5%.

‡Median % change = −17%.

of ara-CTP. However, in either treatment regimen one must consider the possibility that cells that have sustained enough damage to disrupt cellular nucleotide metabolism might lose their bouyancy in the step gradient isolation procedure. For instance, cells whose plasma membrane becomes damaged may not exclude the Ficoll-Hypaque mixture, causing them to sediment to the bottom of the gradient, thus avoiding detection.

The approach that we have described to the study of drug-drug interactions in tumor cells during combination chemotherapy builds on our earlier efforts (18,29). We plan to extend this approach both to investigation of the biochemical modulation of ara-C activity in hematological malignancies and to inquiries into the significance to clinical response of drug-induced DNA damage.

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Simultaneous Cisplatin and 5-Fluorouracil as Second-Line Treatment of Head and Neck Cancer[1]

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Between November 1983 and January 1986, 70 patients with recurrent squamous cell carcinoma of the head and neck were treated with simultaneous cisplatin (20 mg/m^2 for 5 consecutive days) and 5-fluorouracil (400–200 mg/m^2 by iv push for 5 consecutive days) every 3 weeks. Sixty-seven patients were fully evaluable. The major toxic effect was myelosuppression, and two patients died from septic or hemorrhagic complications. Other toxic effects were moderate and quite rare, including nausea and vomiting. All of the patients were treated in the outpatient clinic. The overall response rate was 52.2% (14 complete responses + 21 partial responses). The worst prognostic factor in the present series was the persistence of disease after front-line therapy. This treatment has shown an acceptable toxicity and its antitumoral activity seems to be in the range of the higher activity reported by literature in these kind of patients. [Cancer Treat Rep 71:485–488, 1987]

In patients with head and neck squamous cell carcinoma, response rates to cisplatin range from 16% to 39% (1,2), while for 5-fluorouracil (5-FU) a cumulative response rate of 15% is reported (3). However, the combination of cisplatin and 5-FU by continuous infusion seems to be synergistic: Decker et al (4) reported a response rate of 89% in previously untreated patients and Kish et al (5) observed a response rate of 70% in previously treated patients with advanced disease. Recently, Rowland et al (6) reported a response rate of 60% in patients with advanced, recurrent head and neck cancer using a very similar combination.

However, in a previous experience of our group, we achieved a similar response rate (59.2% in a series of 30 patients with recurrent disease) with a different outpatient schedule using the same drugs (7).

The current pilot study was undertaken to confirm this degree of activity reported by simultaneous administration of bolus cisplatin and 5-FU for 5 consecutive days in patients with advanced, recurrent head and neck squamous cell carcinoma.

PATIENTS AND METHODS

Between November 1983 and January 1986, 70 consecutive patients with recurrent, histologically proven squamous cell head and neck carcinoma were entered in the study. All of these patients but one had had previous treatments, including chemotherapy in 28 patients and two or three different treatments in 52. Three patients were lost to follow-up after the first chemotherapy course and are not considered in this analysis. These three patients include the only patient without any prior treatment, one with persistent disease after radiation therapy, and one with relapse after chemotherapy and radiation therapy. Characteristics of the 67 evaluable patients are shown in table 1.

At the time of their enrollment, 25 patients had failed to respond to first-line treatment (radiation therapy in four patients; radiation therapy and chemotherapy in six; surgery in two; radiation therapy, surgery, and chemotherapy in five; surgery and radiation therapy in seven; and chemotherapy alone in one) and 42 patients had had a relapse after first-line therapy (four received surgery; four received radiation therapy; 18 received surgery and radiation therapy; six received radiation therapy and chemotherapy; and ten received chemotherapy, radiation therapy, and surgery). Previous chemotherapy consisted of vinblastine (6 mg/m^2), bleomycin (30 IU), and methotrexate (200 mg) in all patients. Patient characteristics are shown in table 1. Disease was

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TABLE 1.—Characteristics of evaluable patients

Characteristic	No. of patients	Response		
		CR	PR	None
Male/female	13/54			
Median age in yrs (range): 60 (34-76)				
Median PS (range)*: 1 (0-3)				
Site of primary				
Oral cavity	12	1	2	9
Nasopharynx	5	2	1	2
Oropharynx	23	4	10	9
Hypopharynx	6	1	3	2
Larynx	16	4	4	8
Maxillary sinus	5	2	1	2
Persistent disease	25	4	3	18
Site of persistence				
Local	12	2	2	8
Nodal	5	1	1	3
Local + nodal	8	1	—	7
Relapsed disease	42	10	18	14
Site of relapse				
Local	21	8	4	9
Nodal	6	1	4	1
Distant	3	1	2	—
Local + nodal	12	—	8	4

*ECOG scale.

local in 33 patients, nodal in 11, local plus nodal in 20, and disseminated in three. Forty-three patients had measurable disease and 24 had evaluable disease. Eligibility required adequate bone marrow (wbc count > 3500/mm^3; platelet count > 100,000/mm^3) and renal (creatinine < 1.5 mg/dl) functions. Only patients with Eastern Cooperative Oncology Group performance score (ECOG PS) < 4 were admitted to the study. Pretreatment evaluation included physical examination, chest x-ray, and other relevant scans or roentgenograms. Informed consent was obtained and histological confirmation of diagnosis was required. The chemotherapy schedule was cisplatin (CDDP) (20 mg/m^2 for 5 consecutive days) administered after prehydration (500 ml of 5% dextrose + 5 mEq/L of KCl over a 30-minute infusion) if diuresis was at least 200 ml over 30 minutes. Posthydration consisted of 1.5 L of 5% dextrose plus 5 mEq/L of KCl. Methylprednisolone (125 mg iv) was given at the time of CDDP administration to control nausea and vomiting. 5-FU was administered by iv push at the end of posthydration. Initially, the dose was 400 mg/m^2 but was reduced to 200 mg/m^2 due to hematological toxicity encountered in the first 15 patients. Criteria for response were as follows: *complete response (CR)*—the complete disappearance of all clinically detectable disease; *partial response (PR)*—a 50% reduction in the sum of the products of the longest diameters of all measurable lesions, without appearance of new lesions; *stable disease*—a < 50% reduction or no change in tumor measurement; and *progressive disease*—a > 25% increase or the occurrence of any new lesion. In evaluable patients, PR was defined as an improvement of two grades or normalization in ECOG PS scale, with a grossly reduced mass. Tumor measurements and hematological and biochemical parameters were obtained before each course of chemotherapy.

Study design foresees a maximum CDDP dose of 700 mg/m^2 (seven courses) to prevent neurotoxicity; only patients with continuous improvement after every cycle reached the seventh course of treatment. In other patients, treatment was interrupted after stabilization of response; however, the minimum treatment consisted of four chemotherapy cycles, except in the case of progressive disease. After disease progression, no alternative antiblastic treatments were given.

RESULTS

At the time of the present analysis, follow-up ranges from 1 to 28 months. Five patients developed severe myelotoxicity (two deaths) and stopped treatment. All of these patients received the higher dose of 5-FU (400 mg/m^2); they were considered nonresponders (failure due to toxicity) and were fully evaluated in the analysis.

One to seven cycles of chemotherapy (median, four) were administered. All of the responses appeared within three cycles of therapy. The overall response rate was 52.2%: 20.9% CR rate (14 patients) and 31.3% PR rate (21 patients: 14 with measurable lesions and seven with evaluable lesions). Fifteen patients had stable disease, 12 had disease progression, and five were considered failures due to toxicity. The objective response rate was 57% in patients receiving 5-FU at the dose of 400 mg/m^2 and was 51% in patients receiving the lower dose. The response rate among patients with previous chemotherapy was 57.1%. Of the 25 patients with persistent disease after previous treatment, seven responded (four CRs + three PRs). Of the 42 patients who relapsed after previous treatment, 28 responded (ten CRs + 18 PRs).

The median overall survival was 8 months: 10 months for responders (18 in CR; eight in PR) and 5 months for nonresponders. Survival among patients with persistent disease after first-line treatment is 5 months and among patients with relapsed disease after first-line treatment is 8 months. Disease-free survival in the CR group ranges from 2.5 to 16.5 months (median, 6). At this time, six of the 14 patients with CR are still alive and four are disease-free. Time to disease progression in the PR group ranges from 1 to 23 months (median, 5.5).

Toxic effects are shown in table 2. The initial higher dose of 5-FU (400 mg/m^2) caused severe leukopenia in three patients and thrombocytopenia in three. Two of these patients died from septic or hemorrhagic complications. Transient increases of serum creatinine (range, 1.5–4.0 mg/dl) were observed after administration of ten of 228 courses. Mild stomatitis was observed in four patients. Peripheral neuropathy was noted in a few patients undergoing prolonged therapy.

TABLE 2.—Toxicity

Toxic effect	ECOG criteria for grade—				No. of patients with toxicity at grade—			
	1	2	3	4	1	2	3	4
Nausea and vomiting	Mild	Controllable	Intractable	—	20	14	2	—
Mucositis	Erythema	Localized ulcers	Confluent ulcers	—	4	4	0	—
Renal creatinine (mg/dl)	1.4–2.1	2.1–4.0	> 4.0	Uremia	7	5	0	0
Leukopenia ($\times 10^3$ cells/mm^3)	3.0–3.9	2.0–3.0	1.0–2.0	< 1.0	15	13	3	3
Thrombocytopenia ($\times 10^3$ cells/mm^3)	90–100	50–90	25–50	< 25	5	3	2	3
Hemoglobin (g/dl)	9.4–10.9	8.0–9.4	6.5–7.9	< 6.5	0	0	0	0

DISCUSSION

The palliative role of chemotherapy in the treatment of relapsed or advanced epidermoid carcinoma of the head and neck has expanded with the development of CDDP-based combinations. As early as 1971, Speer et al (8) reported a synergism between CDDP and 5-FU. Moreover, 5-FU toxicity appears to be potentiated by CDDP (9) and correlates with the administration schedule.

The major toxic effect of 5-FU is myelosuppression. This is reduced when this drug is given by continuous infusion, although this route of administration frequently produces severe stomatitis and diarrhea.

To our knowledge, so far only one randomized study on advanced head and neck cancer patients has demonstrated a statistically significant difference in response rate utilizing CDDP and 5-FU infusion versus the same drugs given by iv bolus (overall response rate, 72% vs 20%, respectively) (10). The response rate achieved in the bolus arm was considerably lower than that achieved in our series of patients, although the schedule used in the above study was very different from that employed in ours.

As an extension of our previous pilot study (7) we have utilized the combination of CDDP and 5-FU given simultaneously by iv push for 5 consecutive days every 3 weeks. This schedule has shown an overall response rate of 52.2%, low toxicity, and the great advantage of being easily adaptable to outpatients. With this modality of administration we did not observe any severe stomatitis, but the initial dose of 5-FU (400 mg/m^2 for 5 consecutive days) proved to be too toxic: five of 15 patients experienced life-threatening leukopenia and/or thrombocytopenia. As a result, the 5-FU dose was reduced to 200 mg/m^2 when treatment was well-tolerated and efficacy maintained. Although the response rate is encouraging, the median survival in the current study is only 8 months, quite similar to that reported by others (5,6,11,12).

The failure of second-line therapy to modify survival of these patients has been previously noted (13). These data support the hypothesis that the great majority of pretreated head and neck tumors already contains variable proportions of chemotherapy-resistant cells which are responsible for rapid progression and finally death, despite the initial promising tumor cell killing. The only subset of patients who experience a longer survival of higher quality is represented by patients who achieve CR.

In our study we observed a 20.9% CR rate, data comparable with those achieved by other investigators (5,6) using the same drug combination given by continuous infusion. Antitumoral activity observed in our study must also be evaluated considering the characteristics of our patients: all previously treated and most very extensively treated. However, the major factor affecting the probability of achieving a response in this series was the presence of disease persistence after front-line therapy (worst prognosis) versus disease relapsing after a disease-free interval rather than quality or quantity of previous treatments. We think that this prognostic factor should be stressed in designing new randomized trials.

In our study the simultaneous administration of CDDP and 5-FU has shown acceptable toxicity and demonstrated substantial antitumor activity. Unfortunately, the prognosis of patients with advanced or recurrent head and neck cancer remains very dismal. We are attempting to improve CR rates with various modifications in our current chemotherapy scheme.

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Phase I Study of Two Schedules of Teroxirone[1,2]

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Two schedules of teroxirone, a triazine triepoxide, were evaluated in a phase I study. Twenty-six patients were treated on 1 day every 5 weeks at doses of 36–2250 mg/m^2. At doses $\geq$ 1500 mg/m^2, severe thrombophlebitis was seen without cytotoxic effect, and this schedule was closed. Twenty-seven patients were treated on 5 days every 5 weeks at daily doses of 16–450 mg/m^2. Mild thrombophlebitis and moderate leukopenia were encountered. For phase II studies, a dose of 375 mg/m^2 × 5 every 5 weeks is recommended. Pharmacologic studies showed rapid plasma elimination, which suggests the agent's possible usefulness for regional infusion. [Cancer Treat Rep 71:489–492, 1987]

Teroxirone (Henkel's compound), a triazine triepoxide, exists as two stereoisomers with different physical properties showing antitumor activity against different model tumors, including L1210 and P388 leukemias (1,2). The isomer was chosen for development because of its greater solubility and greater activity. The activity of the isomer (teroxirone) against B16 melanoma and L1210 leukemias and other tumors has been studied in detail (3,4). Cytotoxicity caused by teroxirone is believed to be through its alkylating activity. On all schedules tested, antitumor activity was seen in mice implanted with L1210 leukemia. Teroxirone was most effective on multiple-day schedules compared to single-day or intermittent schedules. Daily treatment for 5 or 9 days increased life span 650%, the maximum increase in life span achievable in this study. Toxicology studies in the mouse and in the beagle dog revealed diarrhea; hypoactivity; weight loss; and evidence of anemia, thrombocytopenia, and leukopenia.

We conducted a phase I study of teroxirone to determine the toxicity and the maximum tolerated dose of this drug given iv on two different schedules: 1 day every 5 weeks and daily × 5 every 5 weeks. We looked for evidence of antitumor response with teroxirone in patients with advanced carcinoma and studied the plasma elimination and urinary excretion.

PATIENTS AND METHODS

Patients

All patients had histologic or cytologic confirmation of unresectable cancer for which there was no conventional means of therapy that offered reasonable hope of cure or of significant palliation. All patients were required to be ambulatory and to be maintaining a reasonable state of nutrition. Patients were excluded for the following reasons: wbc count < 4100/mm^3, platelet count < 130,000/mm^3, hemoglobin < 10 g/dl, any elevation of direct-reacting serum bilirubin, serum creatinine > 1.5 mg/dl, major surgery within the preceding 30 days, prior therapy with cytotoxic agents without full recovery from toxic effects from therapy (20 days from last day of cytotoxic therapy unless nitrosourea or mitomycin, for which 40 days were required), uncontrolled infection, radiation therapy to > 15% of the bone marrow within 30 days or to > 30% of the bone marrow with greater than or equal to the equivalent of 1500 rads in three fractions.

Tests done prior to entry into study included a history, physical examination and tumor measurement whenever possible, wbc count, hemoglobin, platelet count, SMA-12, chest x-ray, electrocardiogram, urinalysis, and iothalamate renal clearance. All patients were seen by a physician prior to each dose of teroxirone and 24 hours after the last treatment on each schedule. Between courses of treatment the hematology group was checked twice weekly and serum creatinine and SGOT were checked weekly. At each level of treatment, patients were entered in groups of three. New patients were entered at a higher dose level only after the three patients treated at the previous level had been observed for a minimum of 3 weeks, counting from the last day of treatment. On both schedules, dose escalations were doubled until toxicity was encountered. At the begin-

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ning signs of toxicity, doses were escalated at 25%. Retreatment of any patient was based on therapeutic response and on the type, severity, duration, and reversibility of any toxic reactions. Any patient showing objective progression of disease or clinical deterioration was not re-treated with teroxirone. No other cytostatic, cytotoxic, or immunostimulatory agent was administered while the patient was in the study. All medications such as analgesics and antiemetics were permitted, as clinically indicated.

Pharmacologic Studies

Venous blood samples were obtained before drug administration, at times after bolus infusion, and during and after continuous infusion of teroxirone. Blood samples were collected into evacuated heparin-containing tubes. Plasma was separated following low-speed centrifugation and stored (−70°C) until analysis. Urine was collected and samples were combined for 24 hours following drug administration. Aliquots were stored (−70°C) until analysis.

Details of the high-performance liquid chromatography (HPLC) assay for teroxirone have been previously described (5). Briefly, teroxirone was isolated from plasma or urine samples (1 ml) by extraction at neutral pH with chloroform (5 ml). Following evaporation of solvent with a stream of nitrogen, the residue was redissolved in 0.01 M pH 7.4 phosphate buffer (0.5 ml) and freshly prepared 5% diethyldithiocarbamate (DDC) to convert teroxirone to the tris-DDC derivative. Following incubation for 1 hour at room temperature, the derivative was extracted with chloroform (5 ml). The concentrated residue was dissolved in toluene for HPLC analysis. Samples were analyzed on a Whatman PAC-CN normal phase column with heptane/ethanol (60/40) as the mobile solvent. Eluting materials were detected by UV absorbance at 254 nm.

Pharmacokinetic data were analyzed using the NONLIN least-squares regression analysis program on a CDC Cyber 170-720 computer. Monoexponential decline in plasma concentrations of teroxirone was fit to the equation $C = Ae^{-xt}$ with a weighting factor of 1/Y. C is the plasma concentration at time t after administration of drug. A is the intercept at $t = 0$, and x is the elimination rate constant. Y is the experimentally determined plasma concentration of teroxirone at time t.

Dose and Schedules of Treatment

Treatment in both schedules was given by rapid iv injection over 10 minutes. To minimize the anticipated phlebitis, the drug was prepared in sterile water and administered by rapid iv injection into a freely flowing iv line containing sterile saline, followed by an additional 50-ml bolus of saline. Because of the thrombophlebitis seen, as discussed in the Results section, this schedule was subsequently altered.

Drug Supply and Preparation

The drug was provided by the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD, as a sterile, freeze-dried powder in 20-ml vials containing 100 mg of drug in 200 mg of mannitol. When constituted with 10 ml of sterile water for injection USP, each milliliter contained 10 mg of teroxirone and 20 mg of mannitol at pH 6.0–7.0 as a clear, colorless solution. The formulated product used in this study was a freeze-dried mixture of teroxirone and mannitol which, unlike a preparation studied previously in Europe, dissolves readily (4).

RESULTS

One-Day Schedule

Twenty-six patients were treated (table 1). The starting dose was 36 mg/m^2 and there was rapid escalation to 1000 mg/m^2. At 1500 mg/m^2, severe thrombophlebitis with cutaneous blistering over the vein was seen, as was

TABLE 1.—1-day schedule

Dose (mg/m^2)	No. of new patients	No. of treatment cycles	Hematologic toxicity: Wbc count nadir < 3000/mm^3		Hematologic toxicity: Platelet count nadir < 85,000/mm^3		Nonhematologic toxicity	
			No. of courses	Range	No. of courses	Range	No. of courses	Description
36	3	3	0		0		0	
72	3	4	0		0		0	
140	2	4	0		0		0	
500	3	3	0		0		0	
1000	3	3	0		0		1	Mild nausea
1500	6	13	4	1200–3000	1	50,000	3	Moderate nausea & vomiting
							9	Moderate to severe phlebitis
1800	6	6	0		1	50,000	3	Moderate nausea & vomiting
2250	0	3	0		0		2	Mild nausea

myelosuppression. Because of phlebitis, the drug preparation was changed from one part drug with three parts 5% dextrose and one-half normal saline to one part drug with six parts 5% dextrose and one-half normal saline; and the administration time was doubled. Although phlebitis was seen, it was not so acute as with the concentrated dose but consisted, instead, of a hard cord with superficial pigmentation over the vein. At the more concentrated infusion solution, we began to see myelosuppression at the 1500-mg/m^2 level, but when the drug was diluted, no myelosuppression was observed. Therefore, we returned to the 1:3 dilution for three more patients at 1800 mg/m^2, giving the drug at two injection sites, one-half dose in each arm. This did not limit thrombophlebitis and produced only mild myelosuppression. One patient was treated at 2250 mg/m^2 three times as part of the continuing dosage escalation scheme in the absence of significant toxicity and stability of disease. Moderate thrombophlebitis without significant myelosuppression was seen after one course of treatment. Because of continued thrombophlebitis, the 1-day schedule was closed.

No tumor responses were seen.

Five-Day Schedule

Twenty-seven patients were treated (table 2). The starting level was 16 mg/m^2 × 5 with rapid escalation to 240 mg/m^2 × 5. At 375 mg/m^2 × 5, myelosuppression was seen in three of six cycles of treatment, and phlebitis was seen in one patient. Eight patients were administered 12 courses of drug at 450 mg/m^2 × 5. Myelosuppression occurred in ten patients with nadir wbc counts ranging from 300 to 2900/mm^3 and nadir platelet counts ranging from 11,000 to 77,000/mm^3. A ninth patient was treated at 450 mg/m^2 × 5 but using the more dilute solution described in the 1-day schedule. No myelosuppression was seen in this patient. At 450 mg/m^2, phlebitis was seen but was not dose limiting. Mild erythema developed along the course of the vein 1 or 2 days during treatment but generally had cleared at the time of re-treatment. Some patients, however, did return with a partially or totally occluded vessel in which the drug had been administered.

No objective responses were seen.

Pharmacologic Studies

Plasma elimination of teroxirone following rapid iv infusion of 140, 240, and 500 mg/m^2 was very rapid. Plasma elimination data (half-life < 2 minutes) were best described by a one-compartment open model with mean values for half-life, total-body clearance, and apparent volume of distribution of 1.4 minutes, 5.7 L/minute, and 12.9 L, respectively. Plasma time-concentration data for four patients who received 240 mg/m^2 are illustrated in figure 1. When teroxirone was administered at constant rates of infusion (12.5–50 mg/minute), plasma concentrations rapidly reached plateau values and remained constant during the infusion. Upon completion of the infusion, plasma concentrations declined as rapidly as after bolus injection. Plasma steady-state concentrations increased as the rate of infusion increased. However, total-body clearance values decreased as the rate of infusion increased.

DISCUSSION

Two schedules of teroxirone were tested in a phase I study. On the 1-day schedule, we encountered severe thrombophlebitis despite drug administration manipulation and concluded that amounts of teroxirone sufficient to produce cytotoxic effects, ie, myelosuppression, without producing severe dose-limiting thrombophlebitis could not be given by peripheral vein in a single infusion. We preferred not to give this drug by central venous line and closed the 1-day schedule.

On the 5-day schedule the myelosuppression at 450 mg/m^2 was somewhat greater than we generally wish to see in solid tumor patients. For phase II studies, we recommend that the dose be reduced to 400 mg/m^2 daily × 5 every 5 weeks. At this dose on this schedule, the drug should be prepared by making a 10-mg/ml solution of teroxirone in sterile water and further diluting

TABLE 2.—5-day schedule

Dose (mg/m^2 × 5)	No. of new patients	No. of treatment cycles	Hematologic activity: Wbc count nadir < 3000/mm^3		Hematologic activity: Platelet count nadir < 85,000/mm^3		Nonhematologic toxicity	
			No. of courses	Range	No. of courses	Range	No. of courses	Description
16	3	3	0		0		0	
32	3	3	0		0		0	
64	2	2	0		0		0	
240	4	10	1	2200	1	68,000	2	Moderate nausea & vomiting
							1	Mild stomatitis
							1	Moderate stomatitis
							3	Moderate diarrhea
300	3	5	1	2500	1	82,000	0	
375	3	6	3	1300-3000	0		1	Moderate phlebitis
450	9	13	10	300-2900	4	11,000–77,000	4	Moderate phlebitis

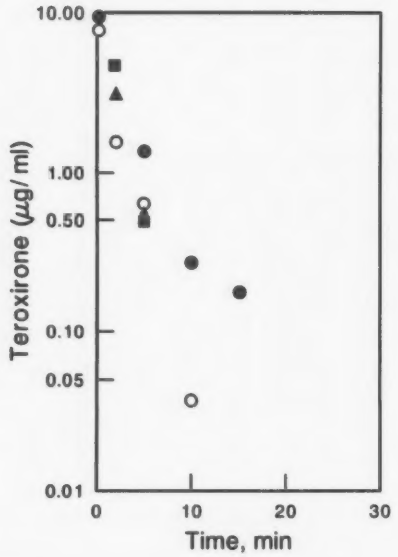

FIGURE 1.—Plasma time-concentration data for 4 patients after administration of 240 mg/m^2 of teroxirone by rapid iv infusion.

that solution to 2.5 mg/ml with either sterile sodium chloride or dextrose solution.

Teroxirone is rapidly eliminated from plasma following bolus administration or following completion of 1–4-hour infusions. We have shown that teroxirone is rapidly metabolized by epoxide hydrase in rat hepatic preparations (5). However, clearance values in patient studies following bolus administration were greater than hepatic blood flow and increased as the rate of infusion was decreased. These data suggest both that there are mechanisms other than hepatic metabolism for plasma elimination and that clearance processes may be saturated when high concentrations of teroxirone are present in blood. Teroxirone is relatively stable in aqueous solution, fresh human whole blood, and fresh human plasma (5).

Teroxirone activity against human tumor cells in culture is abolished when drug and cells are co-incubated with hepatic preparations containing epoxide hydrase (5). This suggests that parent drug is required for activity. Consistent with the hypothesis that teroxirone is an alkylating agent, we found that incubation of teroxirone with human tumor cells leads to formation of DNA-DNA crosslinks (6). The rapid plasma elimination of teroxirone and association of cytotoxic activity with parent drug suggest that teroxirone may have utility as an agent for regional infusion therapy. Ip infusion studies with teroxirone are planned at the National Cancer Institute (J. Collins, personal communication).

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Retinoids as Preventive and Therapeutic Anticancer Agents (Part II)[1,2,3]

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The action of retinoids on tumor initiation and promotion is complex but appears to be carcinogen or cocarcinogen specific (226-229). The large number of newly synthesized retinoids may make it possible to identify analogs that are protective against most or all carcinogens. Many animal model and epidemiologic studies have yielded valuable insights into retinoids' preventive effects (227). The different levels of cancer prevention include primary prevention of neoplastic disease, which occurs before the development of precancerous or cancerous lesions and is accomplished by decreasing or eliminating the exposure to recognized initiating agents (eg, cigarette smoking). The radical-tapping antioxidant action of β-carotene may act to prevent the initiation phase of carcinogenesis. Secondary prevention involves active dietary, pharmacologic, or educational intervention after an individual has been exposed to initiators or promoters but has not yet developed histologically identifiable preneoplastic changes. Retinoids are most effective in the postinitiation portion of carcinogenesis (62,228). They have been postulated to delay the clonal expansion of premalignant initiated cells. In general, retinoids are less effective as the time interval increases between the initial carcinogenic insult and the administration of retinoid therapy (50,229). Tertiary prevention involves the use of dietary or pharmacologic inhibitors in situations where histologically identifiable preneoplastic changes have already developed (23).

At each level of prevention, the physician and patient must evaluate the risk/benefit ratio. For example, a patient who has a serious premalignancy, eg, myelodysplasia, may accept significant toxicity, whereas a patient receiving retinoids to prevent actinic keratosis may not. Clinical investigators must weigh such ethical considerations.

CHEMOPREVENTION OF DERMATOLOGIC CANCERS

Retinoids accumulate preferentially in the skin; therefore, their widest application has been in the management of skin diseases (8,9,11,12,56,207). Many clinical studies have already demonstrated substantial retinoid activity in several types of premalignant skin lesions. The need for controlled studies to define the optimal role for retinoids in cutaneous preneoplasia will become apparent from the following discussion.

Actinic Keratosis

Actinic keratoses are common skin lesions among Caucasians exposed to high levels of ultraviolet light. About 5% of these lesions undergo malignant change and lead to nonmelanomatous skin cancer. Standard treatment consists of local destructive therapy. Impressive prophylactic and therapeutic results have been achieved by treating this condition with topically applied and oral retinoids. Table 1 summarizes the results of the six reported trials.

Belisario (230) has evaluated 93 actinic keratosis patients treated with topical retinoic acid cream. Complete regression occurred in 46 and partial response occurred in 47. Bollag and Ott (231) also used topically applied retinoic acid cream (0.1% and 0.3%) in a 1975 study of this disease. They evaluated 60 lesions, and noted complete regression in 24 (40%) and partial clearing in 27 (45%).

Four studies have employed oral retinoids (232-235). The first by Grupper and Berretti (232) was an uncontrolled trial using etretinate (1 mg/kg/day) for 3 months with subsequent reduction in dose to $\leq$ 0.5 mg/kg/day for 4-5 months. Lesions cleared completely in 75% of the 46 patients and improvement was noted in an additional 20%. Of 34 patients off therapy for 12 months, 26 (76%) relapsed. At present, these authors are treating patients with an annual 2-month intensification course at a daily dose of 1 mg/kg. Most patients remained free of lesions on this schedule. Moriarty et al (233) employed etretinate (75 mg/day for 2 months) in a double-blind, placebo-controlled crossover trial of 50 patients. Of the 44 evaluable patients, 37 (84%) had an objective response (ten complete). Conversely, only two of the 42 placebo-treated

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TABLE 1.—Activity of retinoids in actinic keratosis

Author Reference	Belisario (230)	Bollag & Ott (231)		Grupper & Berretti (232)	Moriarty et al (233)		Watson (234)		Kingston et al (235)
Retinoid	Topical retinoic acid	Topical retinoic acid		Etretinate	Etretinate	Placebo	Etretinate	Placebo	Arotinoid
		0.1%	0.3%						
Evaluable patients	93	26	34	46	44	42	15	15	16
Response									
CR	46	7	17	35	10	1	14	2	0
PR	47	12	15	8	27	1	1	7	10
None	0	7	2	3	7	40	0	6	6

patients responded. Toxicity consisted primarily of scaling and cheilosis, which required dose reductions in 17 patients. Responses occurred within the first month and continued despite dose reductions. No specific information has been provided regarding prevention of subsequent cancers or continued response after the initial treatment period ended. In a recent double-blind study, Watson (234) randomly placed 15 patients with advanced, multiple actinic keratoses on etretinate (1 mg/kg/day) or placebo for 4 months before treatment crossover. Overall, 14 of the 15 patients improved while on the drug. Nine patients received placebo after 4 months on etretinate. One improved, one progressed, and seven remained stable, suggesting a prolonged retinoid effect.

The arotinoid Ro13-6298 was studied by Kingston et al (235), who noted improvement in ten of 16 patients treated with 1 μg/kg/day orally for 28 days. Side effects, including cheilitis, pruritus, plantar-palmar exfoliation, and eczema were seen in 11 cases. There were no significant laboratory abnormalities.

These studies demonstrate the efficacy of retinoids in treating actinic keratoses and indicate that some type of maintenance schedule is necessary for continued response. The high posttherapy relapse rate suggests that subsequent retinoid therapy may be required to prevent disease progression (232). Additional studies are needed to determine the best dosage schedules for both primary and maintenance treatment. Further trials of retinoids in preventing the development of skin cancer are now under way at the University of Arizona and the Memorial Sloan-Kettering Cancer Center (236).

Keratoacanthoma

Keratoacanthomas are hyperkeratotic lesions of unknown etiology which are morphologically similar to squamous cell carcinoma (SCCA) of the skin. They are capable of undergoing either spontaneous regression or, especially when multiple lesions are present, frank malignant change (237).

Preliminary findings in 11 patients suggest that retinoids may have a role in the treatment of this disease. Impressive sustained responses to isotretinoin (2 mg/kg/day) have been reported in five patients with multiple keratoacanthomas (238–241). Six patients experienced complete regression after 2–3 months of oral etretinate (1 mg/kg/day) (232). One year after stopping therapy, five patients have remained in complete remission. As the patients had not experienced spontaneous regression in many years preceding treatment, these cases probably represent the efficacy of the retinoid.

Basal Cell Carcinomas

In addition to the therapeutic effects retinoids have in basal carcinoma (*see* pp 500–501), retinoids also may have a role in preventing the development of these tumors. Peck et al (242) reported three patients who had basal cell carcinomas for 6–16 years before treatment. Based on the number of previous tumors, each patient had a 100% statistical chance of developing new lesions within 1 year (243). More than 2½ years after beginning treatment with isotretinoin, no new tumors had developed. This effect appeared to depend on continued treatment, with an average maintenance dose of 1.5 mg/kg/day. Similar chemopreventive results in other high-risk patients have been reported with both isotretinoin and etretinate (244).

Several large prevention studies are now under way. Basal cell cancer of the skin among albino Africans is the subject of one such study conducted by investigators from Muhimbili Medical Center in Tanzania and the Brigham and Women's Hospital in Boston (236). This pilot trial is examining the effect of β-carotene on the prevention of skin cancer in these high-risk patients. Other investigators are also conducting large, multicenter trials of β-carotene, retinol, and isotretinoin in the secondary prevention of skin cancer.

Epidermodysplasia Verruciformis

This disorder is characterized by familial occurrence, multiple squamous cell skin cancers, and multiple flat, wart-like lesions beginning in childhood. These lesions usually contain human papilloma virus type 5 (HPV-5), which is considered oncogenic (245). The following experience suggests retinoid activity in this disease.

A 32-year-old man with epidermodysplasia verruciformis since age 2 years received etretinate, 1 mg/kg/day orally (246). His lesions regressed within 2 months and on rebiopsy were HPV-5 negative. This regimen has produced similar results in other patients (247,248). Clinical improvement has occurred in some cases de-

spite persistence of the virus (249). Unfortunately, relapse followed dosage reductions. These positive effects may result from an improvement in cell-mediated immunity corresponding to the retinoid treatment (250). The findings in these studies are preliminary, and confirmation must await further trials.

Dysplastic Nevi

The dysplastic nevus syndrome is characterized by the presence of from one to over 100 pigmented macules or flat papules histologically demonstrating dysplastic melanocytes (251). These nevi may occur either familially or sporadically and frequently undergo malignant change to melanoma (252,253). Patients with few lesions are treated with surgical excision; however, if large numbers of nevi are present, treatment is usually withheld unless malignant change appears in a specific lesion on careful photographic follow-up (253). The results of topical therapy with 5-fluorouracil (5-FU) have been variable. We treated three patients with topical tretinoin (0.05% applied daily) (254). After 10–12 weeks, dysplastic histologic features underwent marked reductions to benign nevocellular nevi, a result which has never been reported to occur spontaneously. Currently under way at the University of Arizona are prospective studies involving oral isotretinoin and a randomized trial of topical tretinoin (255).

CHEMOPREVENTION OF NONDERMATOLOGIC CANCERS

Retinoids are currently receiving more attention for prevention of nondermatologic premalignancies. These agents show evidence of activity in many sites, including oral leukoplakia, bronchial metaplasia, laryngeal papillomatosis, cervical dysplasia, myelodysplasia, and recurrent superficial bladder cancer. The results from retinoid studies in these areas are summarized below.

Leukoplakia

Surgical removal of leukoplakia which has become dysplastic may reduce the chance of developing overt malignancy and is considered standard therapy for this condition. Precancerous leukoplakia lesions are not thought to resolve spontaneously and can be safely monitored; therefore, they provide an exceptional opportunity to determine the efficacy of retinoids. In Syrian hamsters, retinyl acetate retards the development of leukoplakia and oral cancer (256). In humans, increased intake of retinol may be associated with a reduced incidence of leukoplakia and possibly of oral cancer (257). Table 2 presents a summary of the six reported studies.

Koch (258) used isotretinoin, tretinoin, or etretinate at 70 mg/day for 8 weeks to treat 90 patients with multifocal and advanced leukoplakia. All had histologic grading and follow-up biopsies. Responses occurred after 3 weeks in a majority of these patients. Eighty-three

TABLE 2.—Activity of retinoids in oral leukoplakia*

Author Reference		Koch (258)			Koch (259)		Cordero et al (260)	Raque et al (261)	Shah et al (262)	Hong et al (263)	
Retinoid		Oral isotretinoin	Tretinoin	Etretinate	Oral etretinate	Oral and topical etretinate	Oral etretinate	Topical isotretinoin	Topical isotretinoin	Oral isotretinoin	Placebo
Evaluable patients		24	27	24	21	24	3	5	11	24	20
Responses (CR + PR)	(%)	87	59	91	71.5	83.5	100	100	82	67	10
Late follow-up (24–30 mos)											
Remission	(%)	45	43	51	52	58	NS	NS	NS	NS	
Relapse or progression	(%)	55	57	51	48	42					
Therapy stopped due to toxicity	(%)	4	11	0	1	1	0	NS	8	8	0

*NS = not stated.

percent (75 of 90 patients) were evaluated 2–6 years after discontinuation of therapy. Follow-up examinations revealed that the etretinate-treated patients experienced higher response rates (91%), more sustained remissions (50%), reduced relapses (46%), and fewer progressions (4%) than did patients treated with either isotretinoin (87%, 44%, 47%, and 9%) or tretinoin (59%, 43%, 40%, and 17%). Relapses occurred within 1 or 2 months after cessation of all treatments, indicating the need for either maintenance or periodic retreatment. Etretinate also seemed more promising in respect to toxicity, as this drug caused no interruptions of therapy while tretinoin and isotretinoin did so in 11% and 4% of patients, respectively.

Based on these initial results, Koch (259) conducted a second study involving 48 patients. For 6 weeks all patients received oral etretinate while 50% were also treated with local application of 0.1% etretinate paste. Both groups had a high response rate within 2 weeks (complete and partial responses = 83.5% in the combined and 71.5% in the oral etretinate-alone group). After 24 months, sustained responses were seen in 58% of the combined-treatment group and 52% of the oral drug-alone group. Relapse occurred within 2 months of stopping therapy. Progression to overt malignancy occurred in four patients (three in the combined group). The authors recommended repeated courses of therapy to reduce relapses. Cordero et al (260) included three patients with oral leukoplakia in a study of etretinate administered orally at an initial dose of 1 mg/kg daily. Partial responses were observed in two patients with grade 2 lesions. A complete remission of an erosive localized lesion was seen in the third patient after 7 weeks of treatment. Another study of five patients produced positive results with topical tretinoin in treating snuff-induced leukoplakia (261).

In a study of 16 patients who received isotretinoin lozenges, responses were demonstrated in all 11 evaluable patients (262). Three complete responses occurred. Response duration was usually short, although there were responses lasting up to 11 months after treatment. In general, toxicity was acceptable, although three of the five inevaluable patients dropped out because of mucositis and cheilitis. In a recent prospective randomized double-blind trial, Hong et al (263,264) also showed that isotretinoin was effective in suppressing leukoplakia. Forty-four patients were evaluated for clinical response after receiving 2 mg/kg/day for 3 months. A complete or partial objective response was seen in 67% (16 of 24) of the drug-treated patients and in only 10% (two of 20) of the patients receiving placebo. Although significant therapeutic response was demonstrated in this study, relapse with progression of lesions usually occurred within 2–3 months after therapy ended.

Thus, retinoids appear to have significant activity in the treatment of leukoplakia. Further studies are needed to determine which analog is most efficacious and least toxic and to establish optimal maintenance treatment guidelines. In addition, investigations of the efficacy of retinoids in preventing second malignancies in patients with a prior history of head and neck cancer should be considered, as should future retinoid trials in patients with hairy leukoplakia and Barrett's esophagus.

Bronchial Metaplasia

Vitamin A deficiency leads to the development of bronchial squamous metaplasia, a precursor of lung cancer (265,266), and administration of vitamin A or its analogs will reverse these changes (3,15–17,267). These observations have generated several studies to determine the effect of retinoids in patients with a high risk of developing lung cancer.

Saccomanno et al (268) reported the use of isotretinoin (1–2.5 mg/kg/day) in 21 patients with abnormal sputum cytology but no evidence of invasive carcinoma. Follow-up sputum cytology, x-rays, and blood studies were performed at 2-week intervals. The retinoid did not alter the cytologic diagnosis in any patient; however, there were degenerative changes of the abnormal cells, suggesting a cytopathic effect of the retinoid. Unfortunately, in light of the 10-year latency period for the development of cancer after metaplastic change, the short treatment period (4–40 weeks) precludes meaningful conclusions.

In 1982, Mathé et al (269,270) reported their experience with oral etretinate (25 mg/day for 6 months) in 52 cigarette smokers with bronchial metaplasia. In each case, bronchoscopy and extensive rebiopsy of previously involved sites were performed after 6 months of treatment. Reductions in the percentages of sections showing metaplasia occurred in 23 of the 30 evaluable patients. Complete resolution of metaplastic changes was noted in three patients who had also stopped smoking. Recognizing the potential for bias, the authors restructured their study to include blinded pathologic review of biopsies (271). Their 1986 survey showed significant results in a study of 40 heavy smokers. Subjects with a $> 15\%$ index of metaplasia received etretinate, 25 mg daily for 6 months. When a second bronchoscopy was performed on the 36 evaluable patients, the index of metaplasia showed a statistically significant decrease from 34.57% to 26.96%. As in the earlier study, four patients who had stopped smoking during therapy demonstrated complete resolutions of their bronchial metaplasia. These results are impressive and suggest that retinoids may play an active role in reversing this preneoplastic process. Confirmation in a large multi-institutional, randomized trial would be an important step toward accepting this approach as standard therapy for heavy smokers (272). Trials employing retinoids to prevent the development of second lung malignancies in patients at high risk should also be considered.

Laryngeal Papillomatosis

Laryngeal papillomatosis is a proliferation of polypoid lesions on the vocal cords, which may precede SCCA. Interferon controls disease in about 50% of these pa-

tients but also produces a considerable degree of toxicity (273–275). Retinoids have been tried because of their antiproliferative activity against transforming epithelial tissues and the lack of any other nontoxic, effective treatment (276).

We used isotretinoin at 1–2 mg/kg/day to treat five patients with recurrent progressive papillomatosis of the larynx (277). Two patients achieved complete regression of their laryngeal lesions, one for 24 months and the other for 36, and a third patient had a partial response lasting 12 months. The two other patients showed no improvement while on retinoids and no additional benefit occurred in these nonresponders when doses were increased. Toxicity was minor and consisted of skin dryness and cheilosis, which were controlled with emollients. Since the time of our initial report, one more patient has been treated and has achieved a complete response. Bichler had similar results employing etretinate (1 mg/kg/day) in 42 patients with laryngeal papillomatosis, who manifested moderate to severe dysplastic changes (278). Twenty-eight patients (67%) achieved complete resolution of their disease and 11 had partial responses.

These results demonstrate that retinoids can control laryngeal papillomatosis and may be preferable to interferon since they are generally less toxic. Further study with longer follow-up is needed to fully evaluate the therapeutic efficacy of retinoids in this disorder.

Cervical Dysplasia

Treatment of cervical dysplasia and carcinoma in situ with retinoids is based on the drugs' successes in reversing preneoplastic epithelial proliferation in other areas of the body and on the epidemiologic association of cervical dysplasia with decreased intake of dietary vitamin A (279,280). The reported intervention studies are summarized in table 3.

Our group has conducted a series of studies employing tretinoin in cervical dysplasia. The initial study of 18 patients utilized a collagen sponge and standard diaphragm to deliver the retinoid. This approach resulted in considerable vaginal toxicity (281). Nevertheless, colposcopy revealed a reduction in size of cervical lesions in six patients, and two were found to have had resolutions of their disease at follow-up conization (282). We subsequently conducted a phase I study in which a cervical cap was used to apply the retinoid (283–285). Thirty-six patients were evaluable after a 4-day regimen of tretinoin cream. Doses were escalated in groups of three, from 0.05% to 0.48% A complete response was experienced by 33% (12 of 36) of the patients. Response was clearly dose-related since 45% (ten of 22) of the complete responders were on high-dose therapy (0.1584%–0.484%), compared to only 14% (two of 14) who received low doses (0.05%–0.1167%). Initial lesion size was not related to disease regression. One of the complete responders with negative biopsies after 12 months had disease recurrence with dysplasia at 18 months.

In a phase II trial at the University of Arizona, we used a tretinoin concentration of 0.372% initially for 4 days and subsequently for 2 days every 3 months over 1 year. Results are available for 20 patients who have been off therapy for at least 6 months (286). A complete response was achieved in ten patients. In most, toxicity with this delivery system was mild to moderate. After 12 months, nine patients remain free of disease.

In a phase I/II study of 50 patients with a histopathologic diagnosis of mild or moderate dysplasia reported by Romney and colleagues (287), placebo or varying dosages of retinyl acetate gel were self-administered using a vaginal applicator for 7 days beginning on the fifth day of the menstrual cycle. Doses ranged from 3 mg to 18 mg. Local and systemic side effects were tolerable at a maximum concentration of 9 mg. Several patients had a decrease in erythema and a general improvement in the appearance of transformation zones, and no patient who completed treatment experienced a progression of her dysplasia.

TABLE 3.—Activity of topical retinoids in cervical dysplasia*

Author	Surwit et al	Weiner et al	Graham et al	Romney et al
Reference	(281)	(285)	(286)	(287)
Preparation	Tretinoin	Tretinoin	Tretinoin	Retinyl acetate
Delivery system	Sponge & diaphragm	Sponge & cap	Sponge & cap	Gel & vaginal applicator
Dose	0.05%–0.2%	0.05%–0.484%	0.372%	Placebo, 3, 6, 9, 18 mg
Patients entered	18	42	25	50
Evaluable patients	18	36	20	40
CR	2†	12	10	NS
PR	4	NS	NS	NS
Colposcopic improvement 50%	6	35	NS	NS
Toxicity				‡
None, mild	5	25	12	
Moderate	3	16	10	
Severe	10	1	1	

*NS = not stated.
†Histologically normal cone biopsy.
‡Moderate toxicity with doses ≥ 9 mg.

While the data appear promising, a definitive statement must await the results and extensive follow-up of our current randomized double-blind phase III trial. Although the results will have important implications for the field of chemoprevention and for the long-term management of cervical preneoplasia, the embriopathic potential of tretinoin and the availability of other successful modalities may limit its clinical utilization for this condition.

Myelodysplastic Syndromes

The myelodysplastic syndromes (MDS) are a heterogeneous group of diseases thought to be caused by clonal proliferation of an abnormal stem cell leading to single or multiple cytopenias (288). Intensive cytotoxic chemotherapy has proved ineffective in controlling MDS, and although initial reports with low-dose cytarabine (Ara-C) were encouraging, recent results have been less so (289, 290). Since approximately 40% of these patients progress to acute leukemia, MDS are considered to be preleukemia states.

Vitamin A deficiency is associated with anemia in humans and with reduced bone marrow precursors in animals (143). Vitamin A replacement corrects these abnormalities. These effects along with the known differentiation-inducing and antiproliferative activities of retinoids opened the door for evaluating retinoids in MDS.

Several in vitro studies have shown that retinoids, especially those with a free terminal carboxyl group such as 13-*cis* and β-all *trans* retinoic acid have potent hematopoietic activity (143). These reports also show conclusively that retinoic acid induces terminal differentiation and cessation of growth of several human leukemic cell lines and marrow cultures from certain patients with MDS and acute myelocytic leukemia (24,41, 291-297). Retinoids enhance clonal proliferation of normal erythroid (CFU-E) and normal myeloid precursors (CFU-GM) in soft gel and increase the responsiveness of these precursors to CSF (296). Retinoids induce HL-60 promyelocytes to lose their proliferative potential, to decrease oncogene expression, and, after 5 days, to differentiate into morphologically mature granulocytes (24,46,297,298). However, cells from less mature leukemic cell lines are often resistant to retinoid-induced differentiation.

These and other in vitro studies documented a differential effect of retinoic acid on normal and neoplastic hematopoietic cells and led to the use of isotretinoin in patients with MDS. The six reported clinical trials of retinoids in MDS are summarized in table 4. In the first series reported by Gold et al (299) in 1983, responses to isotretinoin (20-125 mg/m^2) occurred after 3 weeks of therapy. The response duration could not be ascertained as four of the five responders had discontinued treatment, due to heart failure in one patient, infection in two, and drug intolerance in the fourth. The one remaining responder continued on therapy for 20 weeks before progressing to acute leukemia.

We have also conducted a trial in which the starting dose of isotretinoin was 1.0 mg/kg/day with escalations of 0.5 mg/kg/day (300). Among the responders, one with refractory anemia with ringed sideroblasts (RARS) had an increase of hemoglobin for 4 months, another with RARS had a 50% decrease in red-cell transfusion requirements for 5 months, and one with chronic myelomonocytic leukemia (CMML) had an increase of hemoglobin for 2 months and of granulocytes for 3. Four patients had unexpected drug-induced thrombocytopenia. In another series, 18 patients received a fixed dose of 100 mg/m^2 (301). Responses occurred after 4-6 months of therapy. One patient had a complete response lasting 12 months.

Kerndrup et al (302) treated six patients with 20 mg/m^2 of isotretinoin for 6 weeks. Three received 100 mg/m^2 for an additional 4 weeks. Four patients had a partial response involving several parameters after 3 weeks of therapy. Two of these responses included increases in peripheral blood neutrophils (40% in one, 114% in the other). In three patients, myeloperoxidase-deficient neutrophils disappeared during isotretinoin therapy. An increase in the granulocyte-monocyte colony growth on Day 11 occurred in two responders. Minor cytogenetic abnormalities disappeared in two patients.

TABLE 4.—Activity of retinoids in myelodysplastic syndromes

Author	Gold et al	Greenberg et al	Besa et al	Kerndrup et al	Picozzi et al	Abrahm et al
Reference	(299)	(300)	(301)	(302)	(303)	(304)
Drug	Isotretinoin	Isotretinoin	Isotretinoin	Isotretinoin	Isotretinoin	Isotretinoin
Dose (day)	20-125 mg/m^2	1.0 mg/kg	100 mg/m^2	20 mg/m^2	2.5-4.0 mg/kg	100 mg/m^2
CR	0	0	2	0	1*	1
PR	5	3	2	4	8	0
Patients entered	15	14	18	6	15	1
(FAB subtype)†	(RAEB, 11) (CMML, 4)	(RA, RARS, RAEB, 5; CMML, 2; RAEB, 2)	(NS)‡	(RA, 4)	(NS)‡	(RAEB)

*This patient had a complete clinical and cytogenetic remission; however, the Pelger-Huet anomaly persisted.

†FAB = French-American-British classification; RAEB = refractory anemia with excess blasts; CMML = chronic myelomonocytic leukemia; RA = refractory anemia; RARS = refractory anemia with ringed sideroblasts.

‡NS = not stated.

TABLE 5.—Activity of retinoids in recurrent bladder tumor prevention*

Author Reference	Shaerer et al (314)	NBCCG (315)	Pedersen et al (316)		Alfthan et al (317)		Studer et al (318)	
Retinoid	Etretinate	Isotretinoin	Etretinate	Placebo	Etretinate	Placebo	Etretinate	Placebo
Evaluable patients	5	22†	40	30	15	15	14‡	16‡
Responses (CR + PR)	3	NS	20	26	11	4	NS	NS
Recurrence (%)	NS	NS	NS	NS	60	87	29	56

*NS = not stated.
†Study closed due to prohibitive toxicity.
‡Evaluations at 24 mos.

Recently, Picozzi et al (303) reported the results of 15 MDS patients treated with isotretinoin (2.5–4 mg/kg/day) for 8 weeks. Eight patients (53%) had a > 20% increase in peripheral blood neutrophils and five of these had an absolute increase of > 500. One patient's response included a neutrophil increase from 400 to 2800/cm, normalization of leukocyte alkaline phosphatase score, morphologic improvement in the bone marrow, and disappearance of chromosomal abnormalities. Pelger-Huet cells did persist, however. Abnormalities recurred after cessation of the drug but responded again to its readministration.

Abrahm et al (304) have reported one patient with refractory anemia with excess blasts (RAEB) who had a complete clinical and cytogenetic remission during isotretinoin therapy (100 mg/m^2). After 5 months of therapy, the patient's peripheral blood counts were normal and he was transfusion independent. After 16 months of therapy, the bone marrow returned to normal with < 1% blasts, the peripheral blood counts normalized, and no Pelger-Huet cells or abnormal metaphases were identified. The abnormal marrow growth pattern in vitro remained, however. Isotretinoin was discontinued after 16 months. Seven months later, the bone marrow blasts increased to 28%, Pelger-Huet cells returned, and an abnormal karyotype reappeared. Isotretinoin was restarted at 100 mg/m^2, and 10 months later, the marrow blasts were reduced to < 20% and the Pelger-Huet cells disappeared again. Chromosomal translocations remained, however. Throughout treatment, serial in vitro studies of the patient's marrow growth patterns were conducted. These studies revealed that the isotretinoin had inhibited completely the proliferation of the malignant, monocytic clone, suggesting that the patient's response and prolonged survival were due to this inhibitory effect.

In conclusion, an overall response rate in MDS of 38% (26 of 69 patients), with four well-documented complete responses, indicates that isotretinoin is active in treating this disorder. Clinical evaluation of retinoids with more potent in vitro hematopoietic activity, such as the retinoidal benzoic acid derivative TTNPB (143), should be of interest. Combination therapy with cytotoxic agents or other differentiating agents (such as vitamin D_3, mithramycin, low-dose Ara-C, etc), should be evaluated also. For example, the combination of a DNA synthesis inhibitor (eg, Ara-C or hydroxyurea) plus retinoic acid was more effective than either alone in HL-60 cell lines (305).

Urinary Bladder

In 1972, Evard and Bollag (306) observed a prophylactic effect of tretinoin in human bladder papilloma. Five years later, Sporn and co-workers (307) showed that isotretinoin and etretinate inhibited carcinogen-induced transitional and SCCA of the urinary bladder in rats and mice. The positive retinoid effect occurred after the initiation phase (227). Other studies have correlated low levels of dietary vitamin A with an increased incidence of bladder cancer in humans (19,308,309). Low serum values of retinol, retinol-binding protein, and carotenoid have also been correlated with invasive bladder cancer (309), although these results are subject to alternative explanations. In addition to these data, further rationale for the use of retinoids in bladder cancer has been provided by Fagg et al (310), who identified retinoic acid receptors in over one-half of 43 transurethral resection specimens taken from human bladder tumors.

Clinical trials have included the employment of retinoids following resection of superficial bladder tumors. The expected recurrence rate in this setting is 40%–90% (311–313). The five reported trials are summarized in table 5.

In their 1982 study of etretinate-treated patients, Shaerer et al (314) observed complete tumor disappearance in one case and histologic improvement in two of five patients with recurrent bladder cancers. However, improvement was slow, beginning after 12 months of therapy. The National Bladder Cancer Collaborative Group (NBCCG) initiated a clinical trial involving isotretinoin (0.5 mg/kg/day) in patients with recurrent superficial low-grade lesions (315). Unfortunately, after studying only 22 patients, this trial was prematurely discontinued due to alleged cutaneous and ophthalmologic toxic effects. Although smaller daily doses might still be efficacious and tolerated better, no such evaluation is planned.

A controlled study by Pedersen et al (316) has compared placebo and etretinate (50 mg/day) in 73 patients with recurrent, noninvasive bladder tumor. Cystoscopy was performed every 4 months. Unacceptable mucocutaneous side effects caused 17 etretinate-treated patients to withdraw from the study. Although the prophylactic

effect of etretinate was not significantly different from placebo in this study, the short duration of therapy (8 months) and the large dropout rate (> 50%) in the etretinate group have rendered these results inconclusive.

Using a stratified randomized study design, Alfthan et al (317) reported the results of a placebo-controlled etretinate trial of 32 patients whose recurrent bladder tumors had been removed. Thirty patients were evaluable, 15 of whom had been treated with the drug. After the first ten patients, the initial daily dose was reduced from 50 mg to 25 mg due to side effects. Treatment duration ranged from 10 to 26 months. All patients were evaluated cystoscopically at the start of therapy and reevaluated every 3 months. Compared with the baseline values established for the 2 years preceding the trial, etretinate produced a reduction in the number of tumors per cystoscopy (mean, 2.85 before therapy versus 1.23 after) and was superior to placebo. Eleven patients (73%) in the treated group experienced preventive effects, compared with only four (27%) in the placebo group. Positive etretinate effects were more marked in grade 1-2 tumors than in grade 0 lesions. The recurrence rate for etretinate was 60%, versus 86% for placebo.

A second randomized, placebo-controlled multicenter trial lasting 2 years was conducted in 86 patients with a documented propensity for bladder tumor recurrence (318). As in the first study, dose reductions were required to control the toxicity encountered with 50 mg of etretinate orally. Cystoscopy was performed at 3-month intervals, and tumor recurrence was higher for the placebo-treated patients than for those on the drug after 3 months (40% versus 29%), 12 months (55% versus 35%), and 24 months (56% versus 29%). Recurrences in the placebo group would have been even higher except that nine placebo-treated patients were removed from study by their physicians for lack of efficacy, while only two etretinate-treated patients were removed. Multifocal recurrences were also lower in the etretinate group: 17% versus 48% ($P < 0.02$). Follow-up was limited to 50% of patients who reached the 12- and 24-month evaluation periods. It is of interest that the etretinate-treated patients with the highest number of recurrences reported similar drug toxicity to the placebo-treated patients. With assured compliance, the observed differences might have been more marked.

In summary, based on the positive results from the two randomized double-blind trials, prolonged (> 1 year), low-dose (25 mg/day) etretinate appears effective and compares favorably to therapy with intravesical cytotoxic agents (311-313). These results should be interpreted cautiously, however, because of the small numbers of patients and limited follow-up. Since mucocutaneous toxicity was a major limiting factor, further studies of prolonged low-dose therapy are needed. Also, studies of newer synthetic retinoids having better therapeutic ratios should be encouraged. For example, fenretinide (HPR) is the strongest anticarcinogenic retinoid as indicated by tests in the rodent bladder (319). It is also less toxic in man than are etretinate and isotretinoin, which might allow prolonged therapy with higher doses. A chemopreventive trial of HPR is in progress at the Middlesex Hospital, London (19). Comparing retinoids to intravesical therapy in a randomized trial would be a valuable step toward defining their role in treating this disorder. Preliminary results of an ongoing British trial suggest that retinoids plus thiotepa are synergistic (19). Also, the local instillation of retinoids alone or in conjunction with oral retinoids should be evaluated (320).

RETINOIDS AS THERAPEUTIC ANTICANCER AGENTS

Although retinoids have been viewed classically as cancer prevention agents, considerable laboratory evidence supports their testing as antitumor drugs as well (7,9,11,27,56,128,192). Clinical experience with these compounds in the treatment of established malignancies is increasing and will be reviewed here.

Basal Cell Carcinoma

Topical and systemic retinoids have been used to treat basal cell carcinoma and the reports are summarized in table 6. Early studies employed topical retinoic acid for this disorder and a total of 49 patients were treated by four different groups of investigators. Sixteen complete and 32 partial responses were observed. Bollag and Ott (231) treated 16 patients with topical tretinoin under occlusion at either 0.1% or 0.3%. A complete response was achieved in five patients and a partial response in ten. The therapeutic effect was noted after 6-8 weeks of treatment and occurred with both concentrations of the drug. Follow-up was short, but 10 months after treatment, two of the five complete responders had disease recurrence. Belisario (230) and Schumacher and Stuttgen (321) described similar results with topical retinoic acid in 18 patients, producing a complete response in 50%. Another study of 15 patients by Sankowski et al (322) also demonstrated the therapeutic activity of retinoic acid. Two patients developed a complete response and 13 experienced a decrease in tumor area after treatment with retinoic acid ointment for 21 days. Histologic examination of tumor material from these patients showed cancer cell necrosis after treatments.

Three studies using oral retinoids have also been reported, and their results are summarized in table 6. Peck et al (244,323) used high-dose isotretinoin (4.5 mg/kg/day) and achieved complete clinical and histologic regression in 10% of 248 tumors in 11 patients with multiple basal cell carcinomas. The continued intake of retinoid was necessary for preventing recurrences. Lesions < 10 mm in size responded completely more often than did larger tumors (21% versus 3%). Peck reported improvement in two-thirds of all the lesions and also observed a partial response lasting 16+ months in one of the four study patients with multiple basal cell carcinomas. Several of these lesions resolved completely. Retinoid-induced tumor inflammation was not a prerequisite for response, which suggests that some other

TABLE 6.—Activity of retinoids in basal cell carcinoma

Author	Bollag & Ott (231)	Belisario (230)	Schumacher & Stuttgen (321)	Sankowski et al (322)	Peck et al (323)	Meyskens et al (324)	Grupper & Berretti (232)	
Reference								
Retinoid	Topical tretinoin	Topical tretinoin	Topical tretinoin	Topical tretinoin	Isotretinoin	Isotretinoin	Etretinate	
Evaluable patients	16	12	6	15	11 (248 lesions)	4	20*	20†
Responses								
CR	5	5	4	2	39	0	2	1
PR	10	7	2	13	162	1	7	7
Minor	0	0	0			1	9	5
None	1	0	0	0	47	2	2	7

*Patients with multiple lesions.
†Patients with isolated lesions.

mechanism may have contributed to tumor regression. Patients who discontinued therapy had disease recurrence in 8–18 months. We have also administered isotretinoin to four patients with basal cell carcinoma (324). One partial response was achieved lasting 16+ months. More recently we treated a fifth patient with basal cell carcinoma and vertebral metastases. This patient had a partial response to isotretinoin lasting 6+ months, which has removed the need for major spinal decompression surgery.

Similar experiences with oral etretinate were reported by Grupper and Berretti (232). They treated 40 patients with basal cell carcinomas; about 50% had multiple lesions associated with actinic keratoses and the rest had isolated basal cell cancers. There were 14 initial positive responses, seven in each group. Although the response pattern in each group was similar, relapse patterns differed sharply. Follow-up at 12 months revealed that 12 of 14 patients with multiple lesions had relapsed compared with only one of ten in the isolated neoplasm group. As with isotretinoin, maintenance etretinate appeared necessary, particularly for the patients with multiple lesions. In an interesting case report, an 11-year-old girl with xeroderma pigmentosum who had actinic keratosis and basal cell carcinoma attained a complete response to etretinate (1 mg/kg/day) lasting 7 months, but disease recurred after dose reduction (325).

Although retinoids appear to be effective for the prevention and treatment of cutaneous basal cell carcinoma, we have yet to define the optimal preparation, dose, and treatment schedule. Continuous exposure to the retinoid appears to be necessary to sustain response. These matters may be resolved by the results of several ongoing studies.

Cutaneous Squamous Cell Carcinoma

Currently there is no standard effective systemic therapy for advanced regional, or metastatic SCCA of the skin. Although showing in vivo activity in animals (16,326,327), retinoid treatment for cutaneous SCCA in humans has been limited. As summarized in table 7, four separate studies totalling 14 patients with advanced SCCA of the skin treated with retinoids have been reported. The overall response rate was 71%, including three complete sustained remissions (232,235,324,328).

Grupper and Berretti treated four patients with invasive SCCA and achieved one complete response. They also treated five patients with Bowen's disease and achieved three responses (one complete) (232). Follow-up at 12 months revealed no relapses in the patients with either disease who had been rendered disease-free by retinoids alone or by retinoids along with conventional destructive methods. In a study by Kingston et al (235), five patients with both solar keratosis and SCCA were given arotinoid (Ro13-6298) daily at a dose of 1 μg/kg for 28 days. Although the responses of the four patients with SCCA were not individually described,

TABLE 7.—Activity of systemic retinoids in advanced SCCA of the skin

Author	Grupper & Berretti	Kingston et al	Meyskens et al	Lippman & Meyskens
Reference	(232)	(235)	(324)	(328)
Retinoid	Etretinate	Arotinoid	Isotretinoin	Isotretinoin
Dose/day	1 mg/kg	1 μg/kg	2-3 mg/kg	1 mg/kg
Evaluable patients	4	1	5	4
Response				
CR	1	0	0	2
PR	1	1	3*	2
None	2	0	2	0

*Mixed response.

one patient experienced a dramatic, photographically documented reduction in the size of his lesions after 4 weeks of treatment.

At the University of Arizona, five patients with invasive cutaneous SCCA were studied in a broad phase II trial of isotretinoin (324). Three of these patients had a 50% reduction in some lesions and no change in others (mixed response), and no improvement was seen in the two other patients. Also, we recently used isotretinoin (1 mg/kg/day) to treat four additional patients with advanced, well-differentiated SCCA of the skin after they had failed standard surgical and/or radiation therapy (328). All four patients have tolerated the drug well and two had complete responses lasting 19+ and 3+ months. The two other patients had partial responses of 2 and 9+ months' duration.

These data suggest that retinoids are a highly effective and well-tolerated therapy for advanced SCCA of the skin. As the majority of patients with isolated cutaneous SCCA are cured by local measures, future work with retinoids should be directed to preventing disease recurrence and treatment of patients with advanced local (multiple or surgically difficult) or metastatic lesions. A phase II study of isotretinoin for treatment of advanced SCCA of the skin is now under way at the University of Arizona.

Malignant Eccrine Poroma

This rare neoplasm originates in sweat glands and characteristically runs a chronic course. Roach (329) has reported one patient who had failed surgery, irradiation, and cytotoxic chemotherapy. Within one week after starting isotretinoin (3 mg/kg/day), the lesions became dry and necrotic. Rebiopsy after 9 days of treatment revealed differentiation toward tubules and ducts and some cellular necrosis. This patient had disease recurrence after 8 weeks of tumor response. The experience with retinoids in this tumor is limited to only one other patient, who did not respond (330); however, the impressive, photographically documented response in Roach's patient indicates that isotretinoin should be tried in more of these rare patients.

Melanoma

Early in vitro studies by Lotan and others (29,89) showed that retinoids can suppress growth of cells from human melanoma lines and inhibit growth of murine melanoma and colony-forming human melanoma cells from tumors (30,32,33,40,114,119). Felix et al (331,332) described a significant inhibition of growth resulting from treatment with vitamin A of a transplantable murine melanoma in mice. They also achieved immunological changes in melanoma by treating transplanted melanomas with intralesional vitamin A, which cured 79% of tumors in histoincompatible donor mice. Similarly, topically applied retinoic acid was capable of inhibiting S91 melanoma growth in vivo (218).

In 1980, we demonstrated clinical and pathologic responses in several of two patients' cutaneous melanoma metastases treated topically with 0.05% tretinoin under occlusion (333). Both patients had failed prior cytotoxic chemotherapy. The first patient experienced complete clinical and histologic regression of all of 21 treated lesions and the second had partial regression of disease. The complete responder remained disease free for a total of 18 months after starting therapy, including 1 year after all treatment had ended. Cassidy et al (334) also observed partial responses of cutaneous metastases of melanoma in some patients. These encouraging results prompted inclusion of 20 patients with metastatic melanoma in a broad phase II trial of isotretinoin at the University of Arizona (324). Three partial responses occurred, including a 50% reduction in one patient's 10 × 13-cm subcutaneous mass. These responses are especially noteworthy when one considers the refractory nature of metastatic melanoma. Adjuvant trials of isotretinoin in this disorder are currently under way at the University of Arizona and elsewhere (255).

Further investigation of isotretinoin in metastatic melanoma is needed. Based on in vitro synergy, a two-armed, randomized phase II study of rα-interferon and rα-interferon plus isotretinoin in malignant melanoma is now under way at the University of Arizona.

Mycosis Fungoides

Mycosis fungoides is an uncommon T-cell lymphoproliferative disorder which primarily involves the skin for most of its natural history. Skin involvement often progresses from limited to extensive plaques, then to tumors and generalized erythroderma. After about 8–10 years, visceral involvement becomes clinically apparent. Lymphadenopathy can occur at any time during the clinical course of disease and may represent tumor involvement or reactive dermatopathic change. Currently

TABLE 8.—Activity of systemic retinoids in cutaneous T-cell lymphoma

Author	Kessler et al	Warrell et al	Souteyrand et al (338)		Claudy et al	Molin et al	Zachariae et al
Reference	(336)	(337)			(340)	(341)	(345)
Retinoid	Isotretinoin	Isotretinoin	Etretinate*	Etretinate & PUVA	Etretinate*	Isotretinoin	Etretinate†
Evaluable patients	25	7	5	5	11	28	10
Response							
CR	3	0	2	1	1	6	8
PR	8	3	0	3	7	13	2
Minor	6	0	1	0	3	1	0
None	8	4	2	1	0	8	0

*Includes patients with parapsoriasis-en-plaque.
†Other treatments used simultaneously (*see* text).

available are several topical and systemic treatments, which are often accompanied by unwanted effects, including considerable toxicity, expense, inconvenience, and patient discomfort.

Treatment of this disease with retinoids as single agents has produced impressive results (table 8) (335–344). We have reported previously on our early experience with isotretinoin in four patients with mycosis fungoides (335). We have now treated a total of 25 patients, in which three complete and eight partial responses were documented (336). Median response duration was 8+ months. Six patients had a minor response consisting of a decrease in pruritus and plaque infiltration. Continued retinoid administration has been necessary for maintenance of these responses in our study group. Warrell et al (337) have confirmed our results. In their study of seven patients, three enjoyed excellent responses lasting 7+, 8, and 9 months at an isotretinoin dose of 100 mg/m^2. Responses also occurred in plaque-stage disease, or erythroderma; four patients with tumor-stage mycosis fungoides had no responses. In a study of seven patients with mycosis fungoides, Souteyrand et al (338) treated two with etretinate alone (1 mg/kg/day, reduced after 1 month to 0.7 mg/kg/day). One experienced clinical improvement without accompanying histologic change and the other had no treatment-induced improvement at 1-month follow-up. Three patients with parapsoriasis-en-plaques (considered an early manifestation of mycosis fungoides) were also treated with retinoid alone. Lesions disappeared in two of these cases, after 3 months in one and 4 in the other.

Claudy et al (339) demonstrated the successful use of etretinate in a 77-year-old woman whose advanced, previously untreated cutaneous T-cell lymphoma was limited to the skin. She responded after 1 month and sustained her response for 4 months on continued treatment. Relapse occurred 3 weeks after stopping the drug. Histologic changes corresponded with clinical improvement and then with relapse. Subsequently, Claudy and Rouchouse (340) reported improvement in 11 patients with parapsoriasis-en-plaque treated with etretinate. Clearing of intraepidermal infiltrates including Pautrier's microabscesses was noted. The Scandinavian Mycosis Fungoides Study Group described objective responses in 19 of 28 patients treated with isotretinoin (341). Using an arotinoid, Mahrle et al (342) achieved one partial and three complete responses in five treated patients. Responses to arotinoid occurred in patients with plaque- and tumor-stage disease but not in one patient with Sezary's syndrome.

Wargon and Downie (343) reported favorable results with etretinate in a patient with refractory stage II mycosis fungoides. This patient had progressed despite treatment with PUVA (psoralen + long-wavelength ultraviolet light), steroids, and cytotoxic agents. During the course of these therapies, the patient developed left ventricular failure and widespread recurrent infections. He was started on oral etretinate, 2 mg/kg/day, and experienced almost complete clearing within 3 months and was later placed on an etretinate maintenance dose of 1 mg/kg/day. At 5 months, he remained asymptomatic and without evidence of atypia in skin biopsies of resolved areas. More recently, Fitzpatrick and Mellette (344) reported a 56-year-old man with refractory mycosis fungoides who had complete clearing of his skin lesions after 6 months of isotretinoin (1 mg/kg/day). This patient has remained disease free for 16 months after stopping the drug.

Studies employing combination therapy have also suggested clinical activity of retinoids. Five of the seven patients reported by Souteyrand et al (338) received PUVA in addition to etretinate. Of these, four rapidly improved, including one with a complete response. The fifth patient, who had Sezary's syndrome (erythroderma and circulating neoplastic lymphocytes), showed no improvement. In 1981, Zachariae et al (345) reported treatment results in 16 patients with advanced mycosis fungoides. In conjunction with other treatments, ten patients received etretinate and six did not. The most impressive results were apparent in the retinoid-treated group, in which eight complete and two partial responses were documented. Patients who received etretinate also received cytotoxic antineoplastic agents, PUVA, and prednisone with each treatment cycle. Although the authors considered etretinate to be the most significant factor in this regimen, the study design makes the relative contribution of the retinoid unclear. Hunziker et al (346) and Rusciani et al (347) also obtained favorable results using etretinate combined with methoxypsoralen and PUVA.

These reports demonstrate the need for clinical trials aimed at defining the role of retinoids as alternatives to

aggressive chemotherapy for refractory disease. The therapeutic experiences in mycosis fungoides suggest that retinoids are useful for treating cutaneous T-cell lymphoma. We have begun a trial of isotretinoin plus α-interferon. In addition, we are conducting a phase I trial of whole-body electron beam radiation therapy and isotretinoin. This trial is designed in part to test the feasibility of combining mucocutaneous active agents, such as irradiation and isotretinoin. Whether the beneficial effects derive from immune-mediated events, alteration in target organ response to the abnormal lymphocytes, or a direct effect of retinoids on neoplastic lymphocytes is unclear. Further laboratory investigation of the interaction of retinoids and mycosis fungoides is needed.

Leukemia

Extensive human in vitro and animal in vivo data support the use of retinoids in leukemia, especially of myeloid lineage (24,46,143,294-298). Clinical trials are limited but promising, especially in acute promyelocytic leukemia (APL). In this section we will review in order the in vitro and clinical data for acute nonlymphocytic leukemia (ANLL), lymphoid leukemia/lymphoma, and chronic myelogenous leukemia.

In addition to the cell line studies (eg, HL-60) discussed in the MDS section, several in vitro studies have examined morphological and functional differentiation induced by retinoic acid in bone marrow cells obtained from ANLL patients. Breitman et al (348) found that retinoic acid (10^{-6} M) induced granulocytic differentiation of blast cells from only two of 21 ANLL patients, both of whom had APL (FAB M3) morphology. Similarly, Hofmann et al (349) observed that retinoic acid (10^{-5} M) was relatively inactive as a differentiation-inducing agent in non-M3 ANLL. Honma et al (350) found, however, that retinoic acid induced various degrees of functional and morphologic differentiation in 11 of 12 patients with M2-M5 FAB subtypes. In their study, M2 and M3 ANLL cells differentiated mainly into more mature myeloid cells, and M4 and M5 cells differentiated into monocytes. In addition, they showed that leukemic cells resistant to cytotoxic chemotherapy can be induced to differentiate by retinoic acid (351). Douer and Koeffler (41) showed that retinoic acid inhibited colony formation of leukemic cells from some adults with M1 and M2 morphology but stimulated their normal CFU-GM. In a study by Findley et al (352), retinoic acid (10^{-6} M) inhibited colony and cluster growth of leukemic bone marrow cells in 16 of 19 patients with pediatric ANLL (M1-M5). More recently, Findley et al (353) used a panel of five monoclonal antibodies against normal myeloid differentiation surface antigens to study bone marrow samples from 24 children with ANLL. Sixteen of the 24 samples showed clonal growth in methylcellulose culture. Cultures from ten of these 16 showed retinoic acid (10^{-6} M)-induced inhibition of colony growth, and six of these ten had an increase in myeloid differentiation antigens (after a 5-day incubation with retinoic acid) as detected by T5A7 and 5F1 monoclonal antibodies. Cells from 16 of the children formed colonies and/or clusters and, when incubated with retinoic acid at pharmacologically achievable concentrations, underwent greater reductions of the numbers of colonies and clusters than occurred in control cells not incubated with the drug. Retinoic acid can also induce erythroid differentiation in Friend murine erythroleukemia cells (45,354).

These studies suggest that retinoic acid at achievable in vivo concentrations inhibits clonal growth of leukemic cells and induces an increased expression of maturation-associated myeloid antigens. They also indicate that retinoic acid may cause ANLL blasts to differentiate in vitro into more mature and nonproliferating myeloid cells. All these data provide a strong scientific basis for in vivo study.

Clinical trials of retinoids in ANLL are limited to five case reports of refractory APL, one series of eight adult ANLL patients who were treated with a combination-induction regimen including retinoic acid, and one pediatric series of consolidation vitamin A therapy in eight ANLL patients.

In 1983, Flynn et al (355) described the first APL patient treated with isotretinoin. After a 2-week course of the drug, this patient demonstrated a marked elevation in his peripheral granulocyte count, which correlated with the in vitro response of his cultured bone marrow blasts to isotretinoin. Unfortunately, follow-up was shortened by this patient's death from disseminated candidiasis. Nilsson (356) reported a 30-year-old woman, maintained monthly with Ara-C and 6-thioguanine, whose refractory APL was in relapse for 10 months. She was treated with isotretinoin (1 mg/kg) and began to respond after 1 month. Normal blood and bone marrow counts continued for 11 months. Daenen et al (357) reported a 33-year-old man with APL who failed to respond to treatment with cytarabine, 6-thioguanine, and doxorubicin. His course was complicated by fibrinolysis and *Aspergillus* pneumonia. Due to the latter complication, he was switched to isotretinoin (80 mg/day) as a single agent. After 7 weeks of retinoid therapy, he attained a complete remission lasting 6 months. Fontana et al (358) have recently reported a 27-year-old man with heavily pretreated APL, who had residual disease after multiple courses of chemotherapy. Therapy with isotretinoin (100 mg/m^2) produced a complete response after 13 days. This patient's leukemic blasts differentiated in vitro in response to retinoic acid. Despite continuation of isotretinoin, he relapsed at 1 year. The new population of APL blasts at relapse differed morphologically and were now resistant to retinoic acid-induced differentiation in vitro. Sampi et al (359) reported a 58-year-old Japanese man who also had relapsed APL and was treated with etretinate and dactinomycin. The leukemic cells were sensitive to tretinoin (10^{-6}-10^{-7} M) in vitro, where they showed a synergistic

interaction of differentiation when incubated with dactinomycin. However, the clinical treatment (etretinate plus dactinomycin) did not produce a response. This patient was subsequently treated with a combination of etretinate and cytarabine and again failed to respond. As cells in vitro exhibit a markedly heterogeneous response to different retinoids, possibly the in vitro and in vivo discrepancy results from the use of etretinate in vivo and tretinoin in vitro (359).

Robert et al (360) recently reported a series of eight patients with unfavorable-prognosis ANLL (eg, refractory ANLL, secondary ANLL, old age, and blast crisis) who were induced with cytarabine, isotretinoin (1 mg/kg/day), vitamin D_3, and alpha interferon. There were two complete and three partial responses. Three responders (one complete) did not have marrow hypoplasia consistent with the induction of differentiation. Maintenance therapy consisted of retinoic acid and vitamin D_3.

Lie and Slordahl (361) reported a series of eight children with ANLL who were induced with standard cytarabine, 6-thioguanine, and doxorubicin and were consolidated with high-dose cytarabine plus daily vitamin A, 50,000 IU. Since ANLL in children has a 50%–80% complete response rate but a short duration of response (more than 50% relapse within the first year), this study was aimed at increasing relapse-free survival by use of vitamin A. All eight children are now in continuous, relapse-free survival, ranging from 9–33 months with a mean of 18 months. According to statistics, several of these children should have relapsed within the first year. Although both of these ANLL reports are encouraging, the specific role of the retinoid in induction and maintenance when combined with other agents remains to be determined. However, the results do provide a sufficient basis for a more defined study to test the role of retinoids in prolonging the relapse-free interval in these patients.

Data on retinoid treatment of the lymphoid leukemias are limited. There are few in vitro studies of lymphoid acute leukemia/lymphoma (115), and megakaryoblastic leukemia (M7) has not been studied at all. Retinoic acid (10^{-6} M has inhibited colony growth in four patients with pre-B acute lymphocytic leukemia (ALL), common ALL, and Burkitt's lymphoma lines (352). 13-*cis* and β-all *trans* retinoic acid have induced maturation of leukemic lymphoblasts in vitro from two patients (362). The T-ALL cells from one patient and normal bone marrow CFU-GM were not inhibited at clinically attainable concentrations (352). However, in vitro differentiation of a T-lymphocytic/promyelocytic hybrid along both the myeloid and lymphoid lineage has been reported recently (363). Some recent in vitro data collected by Blakeslee et al (364) suggest that retinol and isotretinoin (10^{-6}–10^{-9} M) inhibited HTLV-1 virus induction (ATLA expression) by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), an alkylating agent, and by 5-iodo-2'-deoxyuridine (IUdR) in a T-cell line derived from a patient with adult T-cell leukemia.

Eighteen patients with indolent, non-Hodgkin's lymphoma or chronic lymphocytic leukemia (CLL) have been treated with isotretinoin at the Memorial Sloan-Kettering Cancer Center (365). Six more lymphoma patients were treated with isotretinoin at the University of Arizona (table 9). Of these 24 patients, only the patient with refractory peripheral T-cell lymphoma had an objective response. Two patients with prolymphocytic leukemia demonstrated a 25% reduction in total peripheral leukocyte count; one stabilized for 6+ months and the other for 10+ months. Warrell et al (366) used retinoic acid (100 mg/m^2/day) to treat an adult with CLL (prolymphocytic) and attained a $>$ 25% reduction in the peripheral lymphocyte count. The activity of isotretinoin in mycosis fungoides and in the patient with peripheral T-cell lymphoma, along with the in vitro data (363,364), suggests the need for further clinical trials of retinoids in advanced T-cell malignancies.

In bone marrow cells from certain CML patients, tretinoin produces a dose-dependent decrease in colony and cluster growth and has induced myeloid differentiation (367–370). Aglietta et al (367) have suggested that retinoids may have value in the management of CML by delaying (or preventing) blastic transformation by inducing differentiation and inhibiting self-renewal. These studies have led to their clinical use in chronic-phase CML (163,369,371). In 1981, Presant and Bearman (371) conducted a small randomized trial with limited follow-up of isotretinoin plus or minus busulfan in 11 patients with chronic-phase CML. In this feasibility study, the drugs were well tolerated. No patient in either treatment group developed blastic transformation at the time of the report. In 1985, Arlin et al (369) treated 17 CML patients with isotretinoin (100 mg/m^2), daunorubicin, Ara-C, and 6-thioguanine. Although toxicity was acceptable, there was no obvious improvement in complete response rates or durations. A large trial with extended follow-up of standard chemotherapy plus or minus isotretinoin is needed to evaluate the role of isotretinoin in delaying or preventing the onset of blast crisis, which was not evaluated in this study. The recent availability of lines from CML blast crisis cells (JOSK-M) may prove useful in evaluating newer retinoids for clinical trials (201).

Certainly, the data do indicate that retinoids have potent antiproliferative and differentiation activity in vitro in certain leukemias. Although the clinical data in APL are limited, the case studies document remarkable responses in four of five previously refractory patients. These results are very encouraging and indicate that more clinical trials are needed to establish the role of retinoids in the therapy of this disorder. Further trials in advanced T-cell lymphoproliferative disorders in addition to mycosis fungoides are also indicated. Combination therapies with other hematologically active biological modifiers (eg, vitamin D_3, interferon, and mithramycin) and/or cytotoxics also deserve further trials. Given its desirably different effects on normal and leukemic progenitors, retinoic acid might also prove useful

TABLE 9.—University of Arizona experience with isotretinoin*

	Total patients	Evaluable patients	CR	PR	MR	SD	PD
Bladder	6	5	0	0	0	1	4
Breast	13	11	0	0	1	2	8
Cervix	11	5	0	0	2	0	3
Cervical dysplasia	85	74	24	4	41	5	0
Choriocarcinoma	6	5	2	0	1	1	1
Colon	23	17	0	0	1	1	15
Esophageal	4	2	0	0	0	1	1
Gastric	2	2	0	0	0	0	2
Head and neck	29	19	0	3	0	0	16
Laryngeal papillomatosis	6	6	3	1	0	0	2
Lung (non-small cell)	33	22	0	0	2	6	14
Lymphoma	7	6	0	0	1	0	5
Melanoma	25	20	0	2	1	2	15
Mycosis fungoides	28	25	3	8	6	0	7
Myelodysplastic syndromes	14	14	0	3	1	7	3
Miscellaneous							
Adenocarcinoma primary unknown	2	2	0	0	0	0	2
Brain	4	4	0	0	0	0	4
Carcinoid	1	1	0	0	0	0	1
CML	2	1	0	0	0	0	1
Testicular	1	1	0	0	0	0	1
Prostate	1	1	0	0	0	1	0
Other	14	9	0	0	0	1	8
Ovarian	18	15	0	1	0	3	11
Pancreatic	4	2	0	0	0	0	2
Sarcoma	13	10	0	0	0	3	7
Skin							
Basal cell	6	5	0	2	1	1	1
Squamous cell	9	9	1	3	3	2	0
Keratoacanthoma	2	2	1	1	0	0	0
Epidermodysplasia	1	1	0	1	0	0	0
TOTAL	370	296	34	29	62	37	134

*CR = complete response; PR = partial response; MR = minor response; SD = stable disease; PD = progressive disease.

for purging bone marrow in the setting of autologous transplantation in CML.

Squamous Cell Cancer of the Head and Neck

We have used isotretinoin to treat 29 patients with various types of advanced head and neck cancers. Of the 19 evaluable patients, three partial responses have been observed, two of which occurred in patients with nasopharyngeal cancers and the other in the only patient with carcinoma of the epiglottis. In all three cases, response durations were short, lasting only 2 months (324). A randomized, three-institution study comparing isotretinoin with methotrexate has accrued 38 patients with advanced head and neck cancer. Patients were randomized to receive either daily isotretinoin (2 mg/kg) or methotrexate (15 mg/m^2 im) for 3 days every 28 days (372). In the 19 patients receiving methotrexate, one minor response was observed. Of the 19 retinoid-treated patients, one complete, one partial, and one minor response were seen, all of short duration.

A limited number of studies have also evaluated therapy of cancer of the head and neck in patients who have received retinoids simultaneously with either cytotoxic anticancer drugs or irradiation. In 1978, a Japanese group reported impressive results from their 1972–1974 treatment of 48 patients which exploited the known synergistic effects of vitamin A and 5-FU administered before each daily radiotherapy fraction (373). Most patients had locally advanced head and neck cancer, and those with marginal responses to this tripartite regimen underwent surgical salvage. Thirty-three of these patients had laryngeal cancer and an overall 4-year survival rate of 78%, as compared to an overall 5-year survival rate in historical Japanese controls and in patients reported by other investigators of approximately 55%. Fifteen patients were treated for hypopharyngeal cancer and experienced an overall 3-year survival rate of 60%, as compared to an overall 3-year rate of $< 50\%$ in patients treated with surgery or irradiation. In a similar study, 25 patients received vitamin A in conjunction with doxorubicin, 5-FU, bleomycin, and methotrexate (374). The response rate in this uncontrolled study was only comparable to rates which have been achieved with regimens not containing vitamin A. Although the authors felt that drug-induced mucositis was reduced with vitamin A, their study design leaves this question open.

Although these combination studies seem promising, their design makes the exact contribution of vitamin A to response and survival unclear. For example, optimism over results of the Japanese study must be tempered by the small number of patients and the use of inadequate controls without studying the effects of vitamin A plus irradiation and 5-FU plus irradiation separately. The complete response induced by single-agent retinoid is noteworthy and suggests that retinoid activity in head and neck cancer should be evaluated further.

Lung Cancer

Micksche et al (375) reported nine male patients with unresectable SCCA who were treated with vitamin A palmitate or isotretinoin. Only one objective response was demonstrated in a patient with histologically proven cancer who later died and was found to be free of tumor at autopsy. Saccomanno et al (268), in the study discussed earlier, included five patients with inoperable invasive bronchogenic carcinoma. No clinical improvement or change in cytologic atypia resulted from isotretinoin treatment. Our phase II trial included 33 lung cancer patients, 22 of whom were evaluable. Two minor responses, six disease stabilizations, and 14 progressions were noted. In 1982, Weber et al (376) treated 46 patients with SCCA of the lung in a randomized trial of bleomycin and lomustine plus or minus etretinate. Between these two regimens, no statistically significant difference in response rates or durations occurred. These results indicate that isotretinoin is not effective in advanced non-small cell lung cancer.

OTHER TUMOR TYPES

We have used isotretinoin to treat a large number of patients with various malignancies (table 9). Some of these patients have been previously reported (277,285, 286,300,324,336). One partial response occurred in an ovarian cancer patient who had a 50% decrease in a pelvic mass and resolution of ascites. In five patients with minimal-disease choriocarcinoma, two complete responses were documented, which is an impressively high rate of response. One of these has been sustained for 3½ years. Tumor types among the evaluable patients with no objective responses were 17 colon, five bladder, five cervical, 11 breast, ten sarcomas, and a number of others. The majority of these patients had been heavily pretreated. Cassidy et al (377) have also reported negative results in 18 advanced breast cancer patients. No objective responses occurred after 2 months of 0.5–8 mg/kg/day of isotretinoin. Considerable toxicity was encountered in this study and probably resulted from the high dose and rapid escalation schedule. Ziegler et al (378) recently reported a pilot study of isotretinoin (2 mg/kg/day) in six patients with Kaposi's sarcoma associated with AIDS (acquired immune deficiency syndrome). Skin toxicity was excessive and all patients' disease progressed.

Also, no responses occurred among 15 patients with advanced germ cell tumors treated at Memorial Hospital (379). All patients had failed prior cisplatin-containing regimens. Although no objective responses were seen, four patients did have stable disease for at least 8 months. One of these, whose original biopsy revealed pure embryonal carcinoma, had mature teratoma at autopsy. These histologic changes may have resulted from his earlier cytotoxic treatment, although retinoid-induced maturation is an intriguing possibility. Such retinoid-induced changes have been documented in vitro in murine teratocarcinoma cell lines and in vivo in mice (35,380).

SIDE EFFECTS AND TOXICITY

We have known for over 100 years that excessive intake of retinol, or hypervitaminosis A, is harmful to humans. The first and classical reported cases of acute hypervitaminosis A involved Eskimos and Arctic explorers who ingested polar bear or seal liver (5–8 mg of retinol/g of liver) and then acutely developed severe headaches, drowsiness, irritability, nausea, and vomiting. Twelve to 24 hours following ingestion, erythema and desquamation of the face, trunk, palms, and soles developed. All symptoms resolved within 7–10 days.

Differences in storage, distribution, and metabolism account for the different toxic effects of various retinoids (12–15,212,220). Hypervitaminosis A occurs when the intake of retinol exceeds the liver's capacity to remove and store it. Among individual synthetic retinoids, toxic effects may differ significantly in both type and degree and seem to be related to both the specific analog and the underlying disorder.

This discussion will have two main parts: acute (major and minor) toxic effects and chronic (major and minor) toxic effects of the prototype molecule, vitamin A. Estimates of the type and relative frequencies of the toxic signs and symptoms are based roughly on several retrospective tabulations from the literature. Also, the specific toxic effects of the widely used derivatives of vitamin A, including isotretinoin, etretinate, and tretinoin, will be discussed (15,212,381–399).

Major acute toxicity in humans occurs 12–24 hours after ingestion of a dose of at least 350,000 IU of vitamin A by infants, and 1,000,000 IU by adults (381,382). CNS symptoms predominate, including severe headaches (28%) and increased CSF pressure (17%) with papilledema, exophthalmos, and bulging fontanelles in infants (26%). These symptoms may be associated with irritability (19%) and dizziness (17%). Nausea, vomiting, fatigue, lethargy, malaise, weakness, and somnolence all occur in approximately one-third of these patients.

Minor acute side effects are more frequent and well described. In order of decreasing frequency, disorders of the skin include cheilitis (> 90%), dryness and scaling with palmarplantar desquamation, pruritus, dry mucous membranes (occasionally with epistaxis), pigmentation, and brittle nails. Ocular symptoms are also prominent, including conjunctival inflammation (40%), dryness, diplopia, and blurry vision. Other side effects include

gastrointestinal and musculoskeletal complaints such as tenderness of long bones (16%), enlarged liver (16%) or spleen (5%), menstrual changes (3%), edema (10%), and rarely, portal hypertension with ascites.

Laboratory abnormalities include reversible elevations of the following: serum retinol (25%), SGOT, SGPT, alkaline phosphatase, GGT (10%–20%), and erythrocyte sedimentation rate (ESR) (40%). Increased liver function tests have been reported in patients receiving as little as 50,000 IU of vitamin A/day; however, liver damage can occur at much lower vitamin A doses when other hepatotoxins (eg, alcohol or methotrexate) are present (381). Other less common (< 10%) laboratory abnormalities include increases in serum uric acid, calcium, and glucose, and clotting abnormalities, but rarely have these presented clinical problems.

Chronic toxicity results from the ingestion of lower daily amounts of retinoids over months or years. Major specific long-term toxic effects include abnormalities in reproductive function and embryological development, serum lipids, the liver, and the skeletal system. Low or high levels of retinoids have been shown conclusively in animal studies to result in teratogenic effects, including abnormal spermatogenesis and embryonic development. Lipid value changes in animals receiving retinoids include hypertriglyceridemia, often associated with an increase in VLDL, a reduction of HDL, and an increase in cholesterol.

Adequate levels of vitamin A are necessary for normal bone development. Although minor hyperostosis may occur on a subacute basis, complications occur more frequently with long-term use. A partial *Homo erectus* skeleton found in Kenya exhibited the earliest pathologically documented bony changes consistent with chronic hypervitaminosis A. Bone and joint pain and tenderness have occurred in 10%–20% of hypervitaminosis A patients. Periosteal calcification of long bones has been reported in all ages, especially children. Retarded growth of long bones and occasional premature epiphysial closure can also occur. Bony changes appear to be long lasting, though not progressive once the retinoid has been stopped. The long bones of elderly patients (60 years and older) appear less sensitive to retinoids. Cranial hyperostosis is seen in 10% of toxic patients. No clear association has been established between elevated alkaline phosphatase and any bony abnormalities in humans or animals. Although occasionally of symptomatic benefit, the use of nonsteroidal, anti-inflammatory agents in preventing retinoid-induced skeletal changes is controversial. It is conceivable that the ability of retinoids to stimulate bone remodelling may be useful in the management of certain less common primary bone disorders.

Minor chronic side effects are more subtle than acute abnormalities (381). The most prevalent signs of chronic toxicity are usually of minor degree and include dry skin and mucous membranes (50%), cheilitis (40%), musculoskeletal abnormalities (50%–60%), pruritus (35%), hair loss (40%), visual disturbances (30%), and occipital headache (40%). Anorexia, weight loss, edema, fatigue, lassitude, and somnolence have also been reported periodically.

Different oral retinoids have rarely been directly compared to each other in respect to the frequency and severity of adverse effects (400). Compared to natural vitamin A, the synthetic retinoids tend to have less severe and fewer central nervous system and liver side effects. Until the recent approval of etretinate, isotretinoin was the only synthetic retinoid commercially available for systemic use in the United States (7,8,11,12). Strauss et al (388) have summarized the short-term side effects of isotretinoin (1 mg/kg/day) in 150 acne patients and we recently reported the effects of a lower dose of isotretinoin (10 mg/day) in normal volunteers (389). In addition, we and others have reviewed the pharmacokinetics of isotretinoin and described a scale for grading toxicity which has been useful for adjusting doses (385–387,395). No drug-related deaths have been reported, possibly because of the rapid reversal of toxicity once the drug is stopped. However, the following mucocutaneous effects are common: cheilitis (90%), dry mouth/excessive thirst (30%), epistaxis (25%), xerosis/desquamation—especially of the face (30%), pruritus (25%), and infrequently erythema, skin fragility, fingertip peeling, pigmentation changes, and hair loss. Ocular side effects include conjunctivitis (40%), photosensitivity (5%–10%), dry eyes with decreased tolerance to contact lenses, reduced night vision with subnormal dark adaptation responses on electroretinogram (6%), and, rarely, corneal opacities (401). Other side effects include various GI complaints (20%), musculoskeletal problems (15%), and headache/lethargy/fatigue (10%). Seizures with electroencephalographic abnormalities are rare and not clearly drug related. Pseudotumor cerebri has been reported in isolated cases. Laboratory findings include elevated ESR (40%), abnormal hematologic parameters (10%–20%), altered liver function tests (10%), and elevated blood sugar. Lipid abnormalities include elevated triglycerides (25%), elevated VLDL, decreased HDL (15%), and increased cholesterol (7%). The LDL cholesterol/HDL cholesterol ratio has shown significant but reversible increases (402). Therefore, in elderly patients with a history of cardiovascular disease, retinoids should be used cautiously. Elevated creatine phosphokinase with vigorous exercise has occurred, but is of unknown significance. Recently, two of our patients on isotretinoin (1 mg/kg/day) developed markedly elevated but reversible creatine phosphokinase levels, one with associated muscle pain and the other with severe disabling polymyositis.

Concern over the skeletal abnormalities associated with chronic retinoid intake has recently increased. Several studies showed that isotretinoin therapy over a 1-year period for disorders of keratinization (eg, ichthyosis) can produce radiographic changes of hyperostosis, and administration for longer periods may result in disabling symptomatic hyperostosis (403). Cunningham (12) found an 8%–28% incidence of vertebral osteophyte formation in patients taking isotretinoin (2 mg/kg/day) for > 2 years. In one prospective study (384), 75% of the

patients developed small vertebral osteophytes in 12 months on a similar dose. These changes are dose- and time-dependent, may reflect other dietary factors (eg, vitamin D), and have not been reported at doses smaller than 1 mg/kg/day. Diffuse idiopathic skeletal hyperostosis (DISH) has been described in four of nine patients receiving high doses (3 mg/kg) of isotretinoin for > 18 months for refractory ichthyosis (390). These changes are seen most often in patients with disorders of keratinization. Of seven cystic acne patients treated with high-dose (similar to that used for icthyosis) isotretinoin, only one developed asymptomatic skeletal changes. Furthermore, a recent prospective study of 96 acne patients treated with isotretinoin (1–2 mg/kg/day) for 4–5 months revealed asymptomatic minimal spinal hyperostosis in ten with changes similar to DISH (404).

Serious teratogenic effects of isotretinoin have been observed in animals and more recently in humans reported in the U.S. and Canada. Lammer et al (405) recently evaluated 154 pregnancies with fetal exposure to isotretinoin and estimated that a 25-fold increase in the risk of major embryopathic changes accompanies this drug. These changes include spontaneous abortions and a characteristic pattern of malformations involving craniofacial, cardiac, thymic, and central nervous system structures. All malformed infants were exposed to isotretinoin no later than Day 28 of gestation. At subteratogenic doses, retinoids can cause problems in the offspring of rats, including behavioral, cognitive, and emotional abnormalities, that have not yet been documented in human offspring. Patients entering prevention trials must be fully aware of possible risks and must employ contraceptive protection. Furthermore, women of child-bearing age should receive pregnancy testing before entering clinical trials. Since the elimination half-life of isotretinoin following chronic therapy is 12–20 hours, patients are advised to avoid pregnancy for a minimum of one full menstrual cycle after stopping therapy.

Recent reviews have detailed the toxicity and pharmacokinetics of etretinate, which is widely used in Europe and only recently approved in the United States (391,394). As with the other retinoids, dose-related mucocutaneous symptoms predominate. The common clinical findings include cheilitis (78%), palmar-plantar exfoliation (31%), hair loss (25%), dry mucosa (21%), and pruritus (15%). Uniquely, this aromatic retinoid increases cutaneous fragility (20%) and creates a sensation of stickiness of the skin. Compared to isotretinoin, etretinate apparently produces fewer skeletal and lipid abnormalities (400,406). Reports of hepatotoxicity with liver function test abnormalities are increasing and now occur in 8%–40% of etretinate-treated patients (396–398). Two serious, specific problems have occurred with this drug: (*a*) severe hepatotoxicity in psoriatic patients also receiving methotrexate, and (*b*) marked teratogenesis due to the long half-life (80–100 days) after chronic therapy. In animals, prolonged high-dose etretinate caused portal inflammation and hepatic fibrosis, the severity of which was much greater than that produced by similar doses of isotretinoin. On the other hand, recent prospective liver biopsy studies in patients with or without pre-existing liver disease (eg, prior methotrexate and/or alcohol exposure) have failed to identify a consistent toxic liver effect from short- or long-term etretinate (407).

Topical tretinoin (393) commonly causes skin irritation (erythema/desquamation) and reversible hypopigmentation (80%). Toxic effects accompanying oral tretinoin are similar to those with vitamin A, except that serious hepatotoxicity has not been reported since tretinoin is not stored in the liver. Adverse effects include headache and dizziness, which occur more frequently (80%), and dry mucous membranes and epistaxis, which are relatively less common (15%–30%) than with the other retinoids. Other toxic effects include cheilitis (80%), xerosis (50%), lethargy/failure (43%), anorexia (30%), pruritus (23%), increased intraocular pressure (11%), hair loss (10%), mental status changes (10%), and nausea and vomiting (30%).

FUTURE DIRECTIONS

Although retinoids have shown efficacy in several premalignancies and malignancies, their clinical use lags behind the in vitro work, which has unequivocally established the activity of retinoids against many malignant cell types. Recent in vitro studies are accumulating a wealth of data about the structure-function relationships of retinoids and their effects on biological and biochemical regulation. This work should now focus on testing the exciting hypothesis that the PK-C cascade system is the central mechanism of retinoid action. Modern molecular biology techniques will help to clarify the way that retinoids affect modulation (possibly through PK-C) of ODC activity, polyamine biosynthesis, oncogenes, and EGF binding. In vitro reversal of doxorubicin-resistant cells is a new and existing discovery which may lead to a significant role for retinoids in reversing drug resistance in various tumors. The availability of many in vivo and in vitro assay systems, including transformed cell lines, will certainly help to focus future clinical studies by revealing the precise specificities and sensitivities of various retinoids. Underwood's (408) warning that significant clinical advances with retinoids will be limited until the "basic science base of understanding expands" underscores the need for this research.

Since most retinoid-responsive neoplastic processes require low-dose maintenance therapy to prevent relapse, the future of retinoids may depend less on screening for efficacy than on screening for toxic effects. The retinamides, arotinoids, and a wide range of new synthetic compounds with retinoid-like activity are extremely promising candidates for this, as well as for primary treatment (59,221,409). Further direction to clinical research is coming from recent in vitro studies which have documented the additive and synergistic activity of retinoids with hormones, DNA synthesis inhibitors, irradiation, and other micronutrients and

biologic modifiers such as DFMO, interferons (α, β, γ), vitamin D_3, and selenium (40,44,59,227,410–425).

Limited resources and the already high and escalating expense of conducting thorough clinical trials compel us to continue to improve methodologies for the conduct of clinical study. These aspects of chemoprevention trials have been reviewed extensively elsewhere (426). It is essential that basic researchers, epidemiologists, and clinicians maintain among themselves a free flow of information in order to specify which areas are most promising for conducting definitive clinical retinoid trials. Of course, as with study of any new chemopreventive agent, problems of compliance and adequate follow-up must be addressed in selecting trial patient populations. In chemoprevention trials, retinoid doses low enough to limit toxicity will enhance patient compliance, although doses must not be so low as to prevent the appearance of positive effects of active drugs within a reasonable time. It is important that trials achieve a large enough reduction in cancer risk to ensure detection. This concern especially applies to cancer trials, in which long latency may delay the appearance of a protective drug effect for several years. Designing studies with adequate sample sizes and lengths of follow-up will help in this area. In the case of fixed populations, stratification of patients into a highest-risk subgroup can also facilitate the observation of achieved significant effects.

Although the efficacy of retinoids is proved in many cutaneous preneoplastic disorders, future work is needed to establish the best use of retinoids in preventing the onset of cutaneous malignancies. Currently at the University of Arizona Cancer Center, we are conducting two stratified randomized prospective studies to evaluate the efficacy of retinoids in the prevention of cutaneous neoplasia. Begun in 1984, the first study includes subjects whose ten or more actinic keratoses had been removed before we randomized them to receive either placebo or retinol (25,000 IU/day, which is five times the U.S. recommended daily allowance). Our primary goal is to determine the number of new basal and squamous cell carcinomas. Our secondary goal is to determine the number of new actinic keratoses. This study has accrued 1800 of a planned 2400 patients. Also begun in 1984, the second study includes patients whose eight or more prior documented cutaneous squamous or basal cell cancers had been surgically removed before we randomized them to receive placebo, retinol (25,000 IU/day), or isotretinoin (0.1 mg/kg/day) in divided doses. To date, 150 of our planned total of 350 patients have been accrued. Our goal in this second study is also to determine the number of new basal and squamous cell cancers that will develop with and without retinoid treatment.

Several noncutaneous preneoplastic disorders, including MDS, bronchial metaplasia, cervical dysplasia, bladder, and breast cancer, deserve special focus for continuing clinical study. In MDS, attention should be focused on the use of new retinoids with free terminal carboxyl groups such as the potent arotinoids (eg, TTNPB) and on combination therapy with cytotoxic drugs or other differentiating agents. Impressive results from phase II studies of retinoids in bronchial metaplasia suggest the need for a large multi-institutional, randomized trial. Our ongoing phase III trial of topical retinoic acid versus placebo in patients with cervical dysplasia should help clarify the role of retinoids in this disorder. Future chemoprevention studies of superficial bladder cancer should compare retinoids to intravesical therapy, investigate surgery with thiotepa, and evaluate the local instillation of retinoids alone or in conjunction with oral retinoids. Although isotretinoin has not proved effective in treating breast cancer, other retinoids (especially 4HPR) alone and combined with antiestrogens show promise in the primary, preventive, and adjuvant therapy of this malignancy (427–429). The positive retinoid trials in preneoplasias such as SCCA of the skin, lung, and head and neck have important implications for adjuvant therapy. Future studies of other premalignancies with easily measured disease (eg, Barrett's esophagus and colonic polyps) should be studied also.

In addition to continuing studies in prevention, certain areas indicate great promise for future studies of retinoid therapy for established human cancers. Three main areas of focus include advanced SCCA and basal cell carcinoma of the skin, mycosis fungoides, and leukemia. Future work with retinoids in cutaneous basal and SCCA should investigate the neoadjuvant treatment of patients with advanced local (multiple or surgically difficult) lesions. In mycosis fungoides, future trials should employ the arotinoids and attempt to define the role for retinoids in combination therapy. Currently we are testing combinations of isotretinoin with whole-body electron beam irradiation or rα-interferon for treating mycosis fungoides. These studies will evaluate in vivo the synergy documented in vitro between α interferon and retinoic acid and should open future directions for testing the general feasibility of combining mucocutaneous-active agents such as electron beam irradiation and isotretinoin. Extensive human in vitro and animal in vivo work and limited clinical trials pave the way for future trials of retinoids in leukemic disorders, especially in APL. Because only five APL patients have received retinoid treatment, which produced four remarkable responses, a multi-institutional, controlled study of retinoids in this disease certainly deserves a future priority.

Investigation of retinoid effects on generally drug-resistant malignancies, such as other advanced T-cell disorders and choriocarcinoma, is suggested by positive in vitro and limited though promising in vivo data (363,364, and table 9).

Another important area for retinoid trials involves their use as effective but less toxic alternatives in treating disorders with currently accepted standard therapies. Current in vitro data (24,34,46,99,143,160,161,196, 197,294–298,348–353) suggest myeloid leukemias and childhood neuroblastoma for clinical retinoid trials. Of course, nonresponse to retinoids is possible, and prospective patients should be thoroughly informed of the

possible risk/benefit ratio of standard treatment versus alternative treatment with retinoids.

Certainly, much has already been accomplished in establishing a valuable role for retinoids in the prevention and treatment of human cancer. Still a great number of questions remain to be answered by further in vitro research, and the clinical applications for single-agent and combination retinoid therapies are only beginning to be determined. To echo Wolbach and Howe in their milestone vitamin A paper of 1925, work "has been begun" and much more "will be reported later."

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NOTE ADDED IN PROOF

Recent data have confirmed our report of positive results in mycosis fungoides and superficial bladder cancer and negative results in advanced non-small cell lung cancer. In nine other patients with mycosis fungoides treated with retinoids, eight responded, with one complete response [Tousignant J, Raymond GP, and Light MJ: Treatment of cutaneous T-cell lymphoma with the arotinoid Ro 13-6298, J Am Acad Dermatol 16:167–171, 1987; Neely SM, Mehlmauer M, and Feinstein DI: The effect of isotretinoin in six patients with cutaneous T-cell lymphoma, Arch Intern Med 147:529–531, 1987; Ippolito F, and Giacalone B: Sur un cas de mycosis fongoide avec manifestations tumorales traite avec Ro 10-9359 (Tigason), Ann Dermatol Venereol (Paris) 109:65–72, 1982; Jenkinson HA, and Beare, JM: Mycosis fungoides, Br J Dermatol 111(Suppl 26):82–84, 1984]. A randomized trial from Italy including 271 patients showed a significant effect of etretinate in preventing recurrences of superficial bladder cancer (Di Silverio F, Concolino G, Tenaglia R, et al: Retinoids in recurrences of superficial bladder tumors. *In* Proceedings of the Fifth International Conference on Adjuvant Therapy of Cancer, Tucson, AZ, 1987, p 75). Finally, a phase II trial of isotretinoin in advanced non-small cell lung cancer produced only one partial response among 23 evaluable patients (Grunberg SM, and Itri L: Treatment of advanced non-small cell lung cancer with 13-*cis* retinoic acid. *In* Proceedings of the Fifth International Conference on Adjuvant Therapy of Cancer, Tucson, AZ, 1987, p 79).

Indicine *N*-Oxide: Clinical Use of a Pyrrolizidine Alkaloid[1]

Susan A. King,* Matthew Suffness, Brian Leyland-Jones, Daniel F. Hoth, and Peter J. O'Dwyer[2,3]

Indicine *N*-oxide (INO), a pyrrolizidine alkaloid, has been evaluated by the National Cancer Institute as an antineoplastic agent. Pyrrolizidine alkaloids are widely recognized as toxic constituents of a variety of plants in the genera *Senecio, Crotalaria, Heliotropium,* and *Symphytum* (1-7). While some of these alkaloids have demonstrated antitumor activity in experimental systems, their potential use is limited due to acute toxicity (7-10). Many are potent hepatotoxins which cause veno-occlusive disease, hepatomegaly, cirrhosis, and hepatoma in animals (1-7). Ingestion of the alkaloid or plant source is also associated with an equine neurological syndrome, a hemolytic syndrome in sheep, and lesions of the muscle, lung, or kidney in swine and fowl (1-3,6,7). INO was first isolated from *Heliotropium indicum* by Mattocks and co-workers in the early 1960s (11) (fig 1). Nearly 15 years passed before the antitumor activity of this agent was characterized by Kugelman and co-workers (12). Preclinical toxicological evaluation of INO did not reveal the typical hepatotoxicity exhibited by other pyrrolizidine alkaloids. For these reasons, INO was selected for clinical development and trials were initiated in late 1977 (13,14). INO, a myelosuppressive agent, has demonstrated activity in acute leukemia but its use has been associated with severe hepatotoxicity in humans. We review here the clinical progress with this drug and discuss recent advances in analog development.

MECHANISM OF ACTION

The precise mechanism for the antitumor effect of INO has not been identified. Many pyrrolizidine alkaloids are cytotoxic agents that may be metabolized from their free base form via allylic oxidation and dehydration into highly reactive pyrroles (15) (fig 2). The pyrrolic metabolites act chemically as alkylating agents (alkylating either from R_1 or R_2) and their formation has been directly linked with toxicity (16,17). The *N*-oxides are less toxic than the corresponding free bases and are believed to act as alkylating agents only after conversion to free bases and then to pyrroles (18). Thus, if INO acted as an alkylating agent through the classical pyrrole mechanism, extensive conversion to the free base, indicine, would be expected. This metabolic route has been demonstrated in vitro where both gut flora and the hepatic microsomal fraction of rabbits catalyzed the anaerobic reduction of INO to indicine (19). The cytochrome P450 system (20) mediates this reduction and the metabolism can be stimulated by Fe(III)-EDTA complexes, diethylenetriamine pentaacetic acid, and Fe(II) ions (21-23). However, in vivo evaluation of INO metabolism in animal models has demonstrated formation of only minor amounts of indicine or other metabolites; the vast majority of the INO dose is excreted unchanged.

Powis et al (24) have demonstrated that the antitumor effect of INO does not depend on conversion to indicine. In their study (24) and in detailed comparison of antitumor models at the National Cancer Institute (NCI) (data on file), INO was determined to be more active in vivo than indicine. Since indicine is more readily converted to the cytotoxic reactive pyrroles, it is not surprising that indicine was more potently cytotoxic than INO in the in vitro systems (25). Against L1210 in vitro, millimolar concentrations of INO were required for growth inhibition, while indicine at one-fifth the INO concentration yielded the same degree of inhibition (25). Millimolar concentrations of INO may be achieved in the clinic (26). Peak levels of indicine approach only 10^{-5} M, well below levels required for in vitro inhibition (26). As evidence against alkylation, Petry and Sipes (27) have not observed DNA-DNA interstrand crosslinks or DNA-protein crosslinks in asssociation with INO-induced cytotoxicity. However, as will be discussed below, INO produces hepatotoxicity in humans which is typical of classical pyrrolizidine-induced toxicity. Since the well-established mechanism of conversion of *N*-oxides to reactive pyrroles via their free bases is not a significant operative method for INO, an alternate method may exist.

Other molecular mechanisms may also be involved in pyrrolizidine toxicity. Recently a metabolite of monocrotaline containing a partially degraded side chain has

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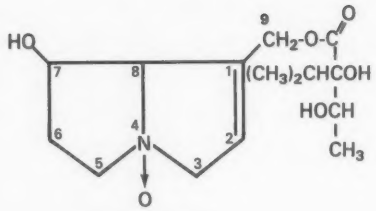
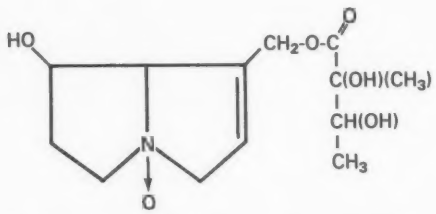
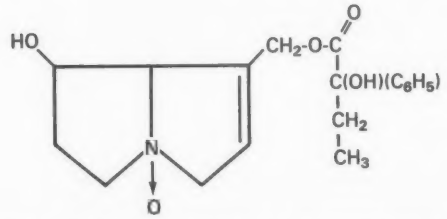


Indicine N-Oxide



9-(2',3'-Dihydroxy-2'-methylbutyrate)retronecine N-Oxide



9-0-[(±)-2-Hydroxy-2-phenylbutyryl]retronecine N-Oxide

FIGURE 1.—Structures of INO and related compounds.

been found to be responsible for the lung toxicity of the parent alkaloid (28); this suggests that chemical transformation of the side chain of the pyrrolizidine alkaloids may be important to their biological activity. In another study, the hepatotoxic compound *trans*-4-hydroxy-2-hexenal has been identified as a metabolite of senecionine, indicating that fragmentation of the pyrrolizidine ring can give rise to reactive species (29). Further investigations of mechanism are clearly required to explain the effects of the pyrrolizidine alkaloids which also have antimitotic and mutagenic properties (30–34). It has been suggested that the *N*-oxide portion of the molecule may be responsible for oxygenation of biologically important compounds, especially purines (35), with possible consequences on DNA conformation (36). In the end, the usefulness of these alkaloids and their analogs will depend upon the ability to separate their antitumor action from their potent hepatotoxicity.

ANTITUMOR ACTIVITY

INO was highly active in vivo against B16 melanoma, P388 leukemia, Walker 256 carcinoma, M5076 sarcoma, and the MX-1 mammary xenograft (3,12,13). To a lesser extent, INO was active against CDF_1 mammary and the L1210 and P1534 leukemias. It was inactive against Colon 26, Colon 38, ependymoblastoma, LX-1 lung xenograft, and the Lewis lung carcinoma (3,12,13). Against cultures of eleven human carcinoma cell lines, INO was minimally active (37). INO's activity in the murine models of L1210 and B16 is superior to that of all other natural pyrrolizidine alkaloids tested to date (12). INO was selected for further development based on its antitumor activity which occurred over a relatively broad therapeutic dose range.

Marked schedule dependency was demonstrated by INO for both B16 and P388; more frequent treatment resulted in greater survival of tumored mice (3,13). Therefore, the daily × 5 schedule was selected for clinical development.

PRECLINICAL TOXICOLOGY

In acute toxicity testing of mice, the LD_{50} and LD_{10} of ip INO were 7270 mg/m^2 and 6000 mg/m^2, respectively (13). Histopathologic evaluation revealed occasional le-

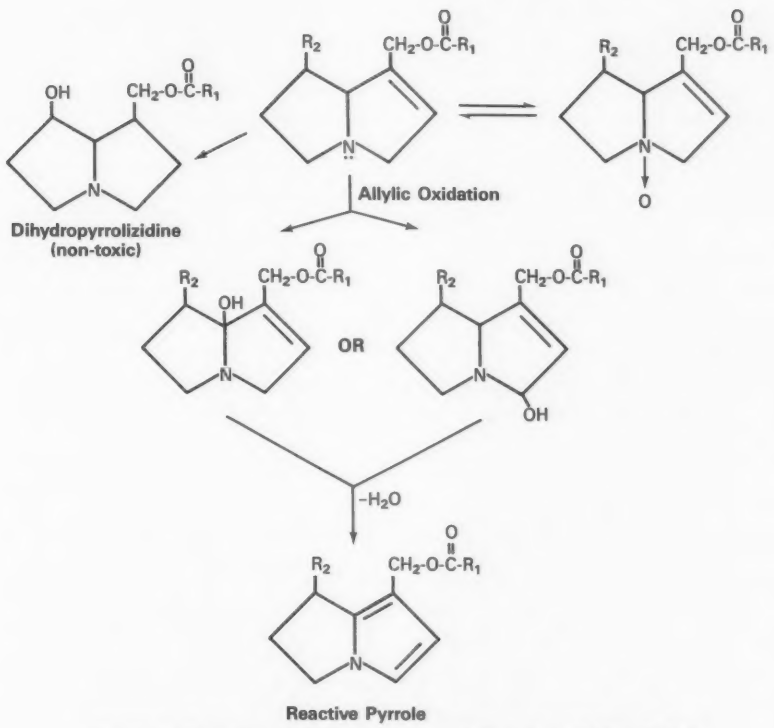


FIGURE 2.—Formation of toxic and nontoxic metabolites of pyrrolizidine alkaloids.

sions of the heart, spleen, kidneys, and duodenum but not in the liver. Dogs and monkeys were treated with various iv doses and schedules of INO. In dogs, a single dose of 24,000 mg/m^2 was lethal. On a daily × 5 schedule, 3000 mg/m^2/day was lethal to dogs and 3600 mg/m^2/day was lethal to rhesus monkeys. Toxicity was noted in several organ systems. Myelosuppression was common with depletion of both myeloid and erythroid elements. Gastrointestinal toxicity, histopathologically seen as mucosal degeneration, correlated with clinical signs of anorexia, emesis (in dogs), diarrhea, and weight loss. The animals showed elevations in BUN and creatinine and histopathologic observations of the kidneys showed toxic nephrosis and the presence of casts in the renal tubules. At the high doses, animals showed altered liver function parameters but histopathologic evaluation revealed only minimal changes (13). In view of the lack of hepatotoxicity, a major concern when dealing with a pyrrolizidine alkaloid, INO was considered appropriate for clinical testing.

PHARMACOLOGY

Methods for the determination of INO and indicine in plasma and urine have been described for electron-capture gas-liquid chromatography (38), gas-liquid chromatography/mass spectrometry (39,40), thin-layer chromatographic colorimetry (41), and differential pulse polarography (42). Radiolabelled drug has been synthesized (43). The most sensitive of these assays can detect nanogram quantities of INO (39,40).

Preclinical Pharmacokinetics

The pharmacokinetic parameters of INO have been studied in mice, rabbits, and monkeys (19,44) (table 1). Both mice and rabbits exhibited biphasic disappearance of INO from serum, while monkeys exhibited triphasic serum disappearance (19,44). Tissue distribution studies following an iv dose of [^{3}H]INO to mice revealed high concentrations of radioactivity in the kidney, liver, and lung tissue at 30 minutes (44). By 120 minutes, the highest concentrations were in the intestine (44). Likewise in the rabbit, iv administered INO found its way to the gastrointestinal tract (19). INO was not actively excreted in the bile and passive diffusion was thought to be the manner by which INO distributes to the gut (19).

INO was rapidly excreted in the urine with only minor conversion to indicine. In both mice and monkeys, 100% of the total radioactivity was excreted within 24 hours with > 80% as unchanged drug (44). Three metabolites were observed in mice. The major metabolite

represented 10%–16% of the dose, while 3% of the dose was represented by the other two metabolites which were thought to be retronecine and indicine (44). In other experiments in mice, 98% of an ip dose of INO was excreted unchanged in the urine, compared to only 27% of an oral dose (24). Oral administration produced a 15-fold increase in the excretion of indicine (24). Rabbits treated iv excreted 65%–83% of the dose within 24 hours, primarily as free and conjugated INO (19). Indicine was detected as a minor constituent throughout the 24-hour collection period (19). Indicine was discovered to be concentrated in the bile 10 times more than in the plasma. While metabolism to indicine was detected in mice and rabbits, the concentration was exceedingly low, approaching a peak plasma level of only 0.6 μg/ml in rabbits after the highest iv dose. The peak plasma concentration of INO in the rabbit was approximately 180 μg/ml (19).

Human Pharmacokinetics

Kovach et al (26) administered INO to 12 adults with advanced cancer as an iv injection for 5 days in doses ranging from 0.45 to 3 g/m^2/day (table 1). Plasma concentrations exhibited a biphasic decline. Peak plasma levels of INO reached 350 μg/ml. Within 24 hours of INO administration, approximately 40% of the total dose was eliminated in the urine as parent compound (10% of which was a nonglucuronide conjugate) and only 2% of the total dose as indicine. Minute quantities of indicine could be detected in plasma after administration of INO doses ≥ 1.0 g/m^2 (25). The terminal half-life decreased with increasing dose of INO. At the highest dose, there was a remarkable increase in the initial distribution phase and a decrease in total body clearance, suggestive of dose-dependent pharmacokinetics (26).

The pharmacokinetic behavior of INO was also evaluated in pediatric patients (45) (table 1). There was no indication of dose-dependent pharmacokinetics over the dose range studied. However, there was evidence of age-dependent pharmacokinetics with the younger children exhibiting decreased plasma elimination half-lives and increased rates of plasma clearance compared with older children. In addition, one patient with renal impairment exhibited a markedly prolonged terminal half-life. Indicine was detected in the plasma of only a few patients at the two highest doses (45).

CLINICAL TRIALS

Phase I

INO was studied in phase I trials on three schedules (table 2). Following evaluation of the daily × 5 schedule in adult cancer patients, Kovach et al (26) found myelosuppression to be the dose-limiting toxicity. This effect appeared to be cumulative; nadirs at the highest dose level occurred around Day 19 for wbc count and Day 18 for platelet count. There were no objective responses among the 29 patients on the trial (26). In a pediatric study using this same schedule, there were two dose-limiting toxic effects—hepatotoxicity and myelosuppression (49). Hepatotoxicity was characterized histopathologically by massive hepatic necrosis and veno-occlusive disease and was implicated in five deaths. Of the 35 children on the study, four complete and three partial responses occurred in 20 leukemia patients [all

TABLE 1.—INO pharmacokinetics

	Method	Dose (iv)	Plasma elimination model	Half-life (mins)			Urinary recovery*	Plasma clearance (ml/min/kg)	Reference
				Alpha	Beta	Gamma			
			Animal model (daily dose × 1)						
BDF_1 mice	Radiolabelled	100–500 mg/kg	Biphasic	10–12	> 100	—	100% (81–87)		(44)
New Zealand rabbit	Electron-capture gas chromatograph	25–111 mg/kg	Biphasic	12.8	70.3	—	65%–83%	16.1	(19)
Rhesus monkey	Radiolabelled	0.24–24 mg/kg	Triphasic	3	32	180	100% (88–96)		(44)
			Humans (daily dose × 5)						
Adult (n = 3 at each level)	Electron-capture gas chromatograph	0.45 g/m^2	Biphasic	3.7	171.7	—	(40)	4.3	(26)
		0.675 g/m^2		1.1	120.7	—		3.6	
		1.0 g/m^2		1.7	101.8	—		6.2	
		1.5 g/m^2		0.8	90.6	—		5.1	
		3.0 g/m^2		40.3†	85.6‡	—		1.8	
Pediatric (n = 21)	Electron-capture gas chromatograph	1.5–4.0 g/m^2	Biphasic	8.0	84.1	—	Not specified	2.1	(45)

*Value in parentheses refers to % of dose recovered as unchanged drug (± conjugate).
†SE = ± 30.0 minutes.
‡SE = ± 41.8 minutes.

TABLE 2.—Phase I trials

Schedule	Dose range (g/m^2/day)	Dose recommended (g/m^2/day)	*N*	Dose-limiting toxicity	Other toxic effects	Reference
			Solid tumors			
Daily × 5	0.15–3.0	3.0	29	Myelosuppression	Nausea and vomiting; hepatic; anemia	(26)
Daily × 5 Pediatric	1.5–3.75	2.0	15	Hepatic; myelosuppression	Nausea and vomiting	(46, 47)
Daily × 1	1.0–9.0	5–7	34	Myelosuppression	Nausea and vomiting; anemia; hepatic	(48)
Daily × 1	5.0–10.0	5–7.5	25	Myelosuppression	Nausea and vomiting; hepatic	(49)
Weekly × 4	1.0–7.5	Not recommended	23	Myelosuppression	Nausea and vomiting; hepatic	(49)
			Leukemia			
Daily × 5 Adult and pediatric	3.0–3.75	Not specified	22	Hepatic	Nausea and vomiting; diarrhea; dermatitis; myelosuppression	(50,51)
Daily × 5 Pediatric	1.5–3.75	2.0	20	Hepatic; myelosuppression	Nausea and vomiting	(46,47)
Daily × 1	5.6–7.5	Not specified	4	Not specified	Hepatic	(52)

responses in acute lymphocytic leukemia (ALL)], and one partial response in glioma among 15 patients with solid tumors (46).

The daily × 5 schedule was also studied in a phase I/II trial in which 22 refractory leukemia patients (adult and pediatric) received initial doses ranging from 3.0 to 3.75 g/m^2/day (50,51). A total of 32 courses were given and mild elevations of SGOT occurred on 20 of these. Five patients developed severe hepatotoxicity (three on their initial course and two on their second course) from which only one recovered (51). Of eight ALL patients on the study, one had a complete remission, while another had a partial remission. Of 11 acute nonlymphocytic leukemia (ANLL) patients, there were two complete remissions; one partial remission was seen among three patients with chronic granulocytic leukemia (CGL) (51).

On the single-dose schedule, myelosuppression was found to be dose-limiting with thrombocytopenia generally more severe than leukopenia (48,49) and nadirs occurring around Day 14 (49). INO was again noted as having cumulative effects on the severity of myelosuppression. Hepatotoxicity was observed in one study where seven of 55 courses were marked by elevations of SGOT, SGPT, and bilirubin (49). In pediatric patients with leukemia on a phase I study of this schedule, INO has been associated with a fatal hepatotoxic event at a dose of 7.5 g/m^2 (52).

One trial using a weekly × 4 schedule was discontinued because of the drug-induced myelosuppression with wbc count and platelet count nadirs occurring around Day 22 and Day 35, respectively (49). The study was modified to an intermittent schedule. Partial responses were noted in liver metastases from colorectal carcinoma in three patients who were originally started on the weekly schedule, two of whom progressed onto the intermittent trial. In addition, one patient with a salivary gland carcinoma had a partial remission as documented by reduction of pulmonary nodules (49).

Phase II

Three phase II studies of INO were initiated. Investigators at the Mayo Clinic studied INO in the treatment of patients with advanced colorectal carcinoma (53). Of 30 evaluable patients, no objective responses were seen but two patients did show some regression of pulmonary nodules. Significant hematologic toxicity was experienced by 12 of the initial 19 patients at doses of 2.25 and 3.0 g/m^2/daily × 5. The study was then restricted to patients with no prior therapy and the dose was reduced to 2.5 g/m^2daily × 5. Only two of 11 patients treated in this manner developed severe myelosuppression. No hepatic toxicity was observed; the only other side effects were nausea and vomiting (53). Of the other two phase II studies, one was conducted by the Children's Cancer Study Group and has recently closed. The other trial, which was conducted by the Southeastern Cancer Study Group, closed soon after opening when hepatotoxicity developed in four of five leukemia patients treated at 3.0 g/m^2/daily × 5 (54).

INO has demonstrated activity against acute leukemia, particularly ALL. In phase I testing alone, INO was associated with five complete remissions and four partial remissions among 23 ALL patients, two complete remissions in 16 ANLL patients, and one partial remission among three with CGL. A full evaluation in phase II could not be performed due to the drug's hepatotoxic effects. Further evaluation of case reports on

hepatotoxicity was conducted at the NCI (NCI files, 1981). Clinically, the hepatotoxicity appears with elevations of bilirubin, SGOT, SGPT, and alkaline phosphatase. Postmortem examinations showed centrilobular necrosis and sinusoidal congestion. Patients with hematologic malignancies experienced greater hepatotoxicity than solid-tumor patients. Based on the case reports, the following conclusions were made regarding the hepatotoxicity of INO: (*a*) hepatotoxicity is dose related, (*b*) no clear evidence exists for cumulative toxicity, and (*c*) risk factors such as prolonged steroid therapy, previous hepatitis, irradiation to the liver, and prolonged oral mercaptopurine (6-MP) and methotrexate (MTX) increase the susceptibility of a patient to INO hepatotoxicity. In July of 1981, patients with these predisposing factors were excluded from study entry. Despite this precaution, the hepatotoxicity of INO was still unpredictable.

DISCUSSION

INO is a naturally occurring pyrrolizidine alkaloid with demonstrated antitumor activity in the murine models. Many of these alkaloids are highly hepatotoxic due to their conversion to reactive pyrroles. INO is generally less toxic than most, possibly due to only minor conversion in vivo to its free base form, indicine. In animal toxicology testing, INO caused hematopoietic and gastrointestinal toxicity, and minor nephrotoxicity. There was virtually no histopathological evidence of hepatotoxicity. Clinical trials of INO began in late 1977. Substantial activity was observed in acute leukemia and there were also scattered hints of solid tumor activity. However, the hepatotoxicity was more severe than expected from preclinical testing. Both hepatotoxicity and antitumor activity were dose-dependent. Because of INO's narrow therapeutic index, efforts are now being directed at developing an analog with a greater therapeutic index.

For analog development, it will be important to determine if modification of INO's structure can selectively enhance antitumor activity while reducing or simply maintaining hepatotoxicity. Presently, it is assumed that the pyrrolizidine ring must remain unsaturated for both these effects (8). Modification of side chains to the ring structure are being investigated by Zalkow et al at the Georgia Institute of Technology (55-57). Substitutions in the ester side chain at the C_1 site are the focus of attention. Adding an aromatic group to the ester moiety attached at the C_1 site (C_9 ester) has resulted in a compound, 9-0-[(±)-2-hydroxy-2-phenylbutyryl]retronecine *N*-oxide (fig 1), with greater in vivo antitumor activity than INO (55-57). A compound with two fewer methyl groups in the C_9 ester than INO, 9-[2′,3′-dihydroxy-2′-methylbutyrate]retronecine *N*-oxide (fig 1), has also demonstrated superior activity (57). This structural approach may have limitations; pyrrolizidine analogs which vary only in their side chains may fail to exhibit an adequate separation of antitumor and hepatotoxic effects, especially if the mechanisms of these effects are the same. For clinical use, the selective enhancement of antitumor activity will have to be of such magnitude that the therapeutic dose level is always well below that which causes hepatotoxicity.

A key problem in development of an improved analog for clinical study is the demonstration of less hepatotoxicity than INO. Thus far, it has not been possible to develop an adequate animal model for INO hepatotoxicity. In the standard models which detect known hepatotoxic pyrrolizidines such as monocrotaline, INO shows no hepatotoxicity. The development of a model which can predict for INO hepatotoxicity is required for comparison of the new analogs.

It would be possible to improve the therapeutic index of INO if hepatotoxicity could be ameliorated or prevented without reduction of the antitumor activity. Conjugation with a monoclonal antibody is a future possibility. In addition, the development of a chemoprotective agent has been hypothesized, especially in view of the ubiquitous nature of the pyrrolizidine alkaloids in the environment. It is known that cysteine protects the rat liver from the toxic effects of the pyrrolizidine alkaloid monocrotaline, but this chemoprotective effect has not been demonstrated in other species (58). Phenobarbitone has been found to protect rats from lasiocarpine toxicity but to increase the toxicity of another pyrrolizidine alkaloid, heliotrine (59). Further advances in this area will depend upon the determination of the etiology and molecular basis of veno-occlusive disease.

The demonstrated therapeutic activity of INO in clinical trials suggests that further investigations of chemoprotective methods and analog development are warranted. Advances in both of these areas will be greatly expedited by the development of in vitro and in vivo models to predict for human hepatotoxicity.

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BRIEF REPORTS

Phase II Trial of Cisplatin and Etoposide in Children With Advanced Soft Tissue Sarcoma: A Report From the Italian Cooperative Rhabdomyosarcoma Group[1,2,3]

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A phase II multicenter evaluation of cisplatin (90 mg/m^2, Day 1) and etoposide (150 mg/m^2, Days 2, 3, and 4) in 4-week cycles was carried out in 21 relapsed children with rhabdomyosarcoma (RMS) and six with non-RMS soft tissue sarcomas (NRSTS). Clinical responses were evaluated after four cycles. There were three complete responses (CRs) and four partial responses (PRs) among the 21 patients with RMS and no responses among the patients with NRSTS. The durations of the three CRs and the four PRs were 11, 9, and 8 months, and 5, 2, 2, and 1 month, respectively. Bone marrow and renal toxicity was cumulative and predictable, but not life threatening. The response rate (CR + PR = 33.3%) to cisplatin and etoposide warrants testing as front-line therapy for RMS. [Cancer Treat Rep 71:525–527, 1987]

Cisplatin (CDDP) has shown considerable activity in a number of pediatric tumors (1). A 15% response rate for CDDP against rhabdomyosarcoma (RMS) and undifferentiated sarcoma has been demonstrated by the Children's Cancer Study Group in a phase II trial (2). Etoposide (VP-16) in phase II studies showed wide-spectrum therapeutic activity against both leukemia-lymphoma and solid tumors (3). In two different series, response rates of 19% and 21% were achieved in intensively pretreated children with RMS (4,5).

The experimentally and clinically demonstrated synergism between VP-16 and CDDP rendered this combination particularly attractive (6), so that we conducted a phase II multicenter clinical trial of VP-16 plus CDDP in children with refractory soft tissue sarcomas.

MATERIALS AND METHODS

A total of 30 patients under 15 years of age with histologically proven soft tissue sarcomas, all resistant to the same previous chemotherapy (cyclophosphamide plus dactinomycin plus vincristine plus doxorubicin), were entered in the study.

Twenty-one patients with RMS and six with non-RMS soft tissue sarcomas (NRSTS) (one malignant fibrous histiocytoma, one angiosarcoma, two fibrosarcoma, one neuroepithelioma, and one synovial sarcoma) met study requirements; ie, presence of measurable disease, normal renal function, recovery from toxicity induced by previous agents, and a life expectancy of at least 6 weeks. Three patients were not evaluable, two because of contemporary radiotherapy and one because of major protocol violations.

Pretreatment investigations included clinical evaluation, measurement of lesions, cbc, hepatic and renal function tests, bone marrow aspirates, and chest x-ray films. Measurement of index lesions as well as hemogram and hepatic and renal function tests was repeated at 2- or 4-week intervals.

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[2] Supported by CNR Special Project "Oncology," grant No. 840049544.
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Treatment Schedule

CDDP was infused over 6 hours in a dosage of 90 mg/m^2 on Day 1, in a volume of 1000 ml/m^2 of 5% dextrose in 0.3% saline with mannitol, 10 g/m^2, and with adequate prehydration 2 hours prior to the CDDP. VP-16 was administered as a 1-hour infusion in a dosage of 150 mg/m^2 on Days 2, 3, and 4. The course of therapy was repeated every 3 or 4 weeks. Supplemental oral magnesium gluconate (2 g/m^2/day) was given starting with the second course.

Evaluation of Response

The antitumor responses were defined as follows: *complete response* (CR) as the disappearance of all measurable disease and *partial response* (PR) as at least a 50% reduction in the product of perpendicular diameters of the lesion maintained for at least 4 weeks. All responses with < 50% regression of tumor were considered nonresponses.

The sample size needed for this study was calculated according to the two-stage patient accrual plan for phase II trials (7).

RESULTS

Responses to Treatment

Three CRs and four PRs were achieved among the 21 children with advanced RMS, while no response was observed in the six children with NRSTS. The three CRs were observed in a boy who showed a complete disappearance of lung metastases after four courses, and in two other patients with pelvic tumor recurrences after six courses of therapy. The durations of response were 11, 9, and 8 months in the three patients with CR. The durations of response were shorter in patients with PR (5, 2, and 1 months). Two months later, one patient with PR obtained a CR with radiation therapy.

Positive responses to the CDDP plus VP-16 combination were observed only in the group of children who relapsed after achieving a CR following treatments with the four drugs previously mentioned (table 1). No positive responses were observed in the eight patients who never obtained a good response to previous chemotherapy and had progressive disease.

TABLE 1.—Responses to CDDP and VP-16 therapy in 21 patients with RMS according to clinical status

	No. of patients	CR	PR	No response
Prior response*				
Relapse "on therapy"	5	2	1	2
Relapse "off therapy"	8	1	3	4
No prior response*				
Progressive disease	8	—	—	8

*To previous treatment with cyclophosphamide, dactinomycin, vincristine, and doxorubicin.

TABLE 2.—Toxic effects of CDDP and VP-16 therapy (data from 24 patients)

Toxic effect	No. of patients
Nausea and vomiting	24
Hematopoietic depression	
Hemoglobin < 8 g/dl	4
Wbc count < 1500/mm^3	7
Platelet count < 50,000/mm^3	5
Infection	2
Stomatitis	2
Renal function abnormalities	
Creatinine > 1 mg/dl	3
Mg < 1.4 mEq/L	9

Toxicity

The toxic effects observed in 24 children with sufficient data are summarized in table 2. All developed nausea and vomiting which was, however, controlled with metoclopramide or chlorpromazine. Myelotoxicity was not severe, requiring dose reduction to two-thirds in three patients and schedule delay for > 7 days in four. Thus, 92 of the 105 courses (87%) administered were delivered at full dosage, while the time interval between the courses ranged from 20 to 48 days (median, 26). Only seven patients had wbc counts < 1500/mm^3 and five had platelet counts < 50,000/mm^3.

Two cases of infection occurred: one was a case of *Staphylococcus epidermidis* sepsis and the other was a case of cystitis. Nephrotoxicity was slight. Four children showed a transitory rise in serum creatinine to a maximum value of 1.5 mg/dl. There was moderate magnesium wasting in nine patients, but no hypocalcemia. In one patient CDDP was withheld because of the appearance of EEG abnormalities just after the first course, which worsened after the second cycle. Serial audiograms were not performed systematically, but no symptomatic impairment of hearing was observed.

DISCUSSION

A 33% response rate to CDDP plus VP-16 with three CRs in 21 patients with advanced RMS is noteworthy. Positive responses were obtained only in patients who relapsed after achieving CR following prior therapy with cyclophosphamide plus vincristine plus dactinomycin plus doxorubicin, whether on or off therapy. Patients who did not display a positive response to the previous treatment did not obtain a CR or PR to the CDDP plus VP-16 combination. The data suggest that the combination of CDDP and VP-16 may be more active than either agent alone.

No favorable responses were obtained in six children with NRSTS. However, the small number of patients

does not allow definitive conclusions to be drawn regarding the effectiveness of the CDDP plus VP-16 combination against such tumors.

Toxicity was moderate: renal toxicity was low and bone marrow depression pronounced in only a few cases, but even then it was easily managed.

We conclude that the CDDP plus VP-16 combination is feasible and effective against RMS, and suggest that it should be tested as front-line therapy in patients with RMS.

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BRIEF REPORTS

Acute Myocardial Effects of Mitoxantrone in the Rabbit[1,2]

Francesca Maria Tumminello, Gaetano Leto, Nicola Gebbia,* Vittorio Gebbia, Anna Russo, and Luciano Rausa[3,4]

Some clinical studies that were performed for the purpose of assessing the potential cardiotoxicity of mitoxantrone (DHAD) have shown that repeated administrations of the drug in some patients cause a mild impairment of cardiac functions and morphological changes in the myocardial cells qualitatively similar to those elicited by anthracyclines. Since doxorubicin has been reported to cause acute cardiac effects, probably related to its chronic cardiotoxicity, experiments were carried out on the rabbit heart to investigate whether DHAD is also able to induce acute cardiac effects. Our results show that this drug caused a reversible dose-related impairment of cardiac contractility on the isolated and perfused rabbit heart while it was not able to induce ECG alterations in normal rabbits. These findings demonstrate that in the rabbit DHAD induces an acute cardiac activity qualitatively similar to that of doxorubicin and suggest that this animal model could be a useful tool to study the cardiac actions and related pathogenetic mechanism(s) of this antitumor drug. [Cancer Treat Rep 71:529-531, 1987]

Some clinical studies report that several patients developed evidence of clinical congestive heart failure during and following therapy with mitoxantrone (DHAD) (1-3), an anthracenedione derivative structurally similar to anthracyclines, which is mainly used in advanced breast cancer (4).

Although early experimental studies suggested that DHAD was devoid of chronic cardiotoxicity (5,6), further investigations performed in an attempt to assess the potential cardiotoxicity of the drug on both animals and humans (2,3,7) have shown that multiple-dose administrations of DHAD are able to induce a mild impairment of cardiac functions and morphological changes in the myocardial cells qualitatively similar to those induced by doxorubicin. Since anthracyclines and other antitumor drugs are often able to elicit acute cardiovascular effects (8-11), which might be related to the mechanism(s) of chronic cardiotoxicity (12), we undertook some experiments on the rabbit heart, which in our experience is the most suitable model (8-10), to investigate the liability of DHAD to cause acute cardiac effects.

MATERIALS AND METHODS

DHAD was supplied by Cyanamid Chemical Co, Catania, Italy. New Zealand male rabbits (1.5-2.5 kg) were used in these experiments.

In vitro studies.—Methods employed in isolating and mounting rabbit hearts have been described in detail (10). In brief, the animals were killed by stunning. The heart was rapidly excised, mounted in a Langendorff apparatus, and perfused in a retrograde manner at a constant pressure and at 37°C with an oxygenated Locke-Ringer solution. Left ventricular pressure (LVP), its first derivative (dP/dt_{max}), and coronary flow were measured as previously described (10). Heart rate was maintained at 60 beats over basal values by electrical stimulation by means of two electrodes inserted into the right atrium and in the ventricular apex, respectively (10). DHAD was given at the concentrations shown in table 1, dissolved in the perfusion fluid; pH value of perfusion fluid was not altered by addition of the drug. Each concentration of the drug was tested on different

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TABLE 1.—Effects of DHAD at given concentrations on LVP and dP/dt_{max}*

Concentration (M)	LVP (% change)	*P* value	dP/dt_{max} (% change)	*P* value
	During DHAD 1st perfusion			
1.5×10^{-6}	−23.8 ± 6.6	< 0.02	−38.3 ± 11.1	< 0.02
2.3×10^{-6}	−32.9 ± 8.8	< 0.02	−41.9 ± 11.6	< 0.01
3.1×10^{-6}	−35.7 ± 10.3	< 0.01	−45.5 ± 10.7	< 0.01
3.9×10^{-6}	−43.2 ± 15.9	< 0.05	−47.7 ± 14.7	< 0.05
	After stopping DHAD perfusion			
1.5×10^{-6}	−1.2 ± 8.1	NS	−9.1 ± 4.5	NS
2.3×10^{-6}	−5.9 ± 2.2	NS	−4.4 ± 4.6	NS
3.1×10^{-6}	−6.4 ± 3.7	NS	−11.4 ± 3.4	NS
3.9×10^{-6}	−0.4 ± 6.2	NS	−3.1 ± 2.8	NS
	During DHAD 2nd perfusion			
1.5×10^{-6}	−2.9 ± 6.4	NS	−14.1 ± 5.6	NS
2.3×10^{-6}	−6.6 ± 3.4	NS	−5.3 ± 5.0	NS
3.1×10^{-6}	−22.9 ± 6.9	< 0.02	−28.7 ± 9.9	< 0.01
3.9×10^{-6}	−14.3 ± 6.9	< 0.05	−20.3 ± 5.6	< 0.05
	After stopping DHAD perfusion			
1.5×10^{-6}	−1.8 ± 5.8	NS	−0.5 ± 16.9	NS
2.3×10^{-6}	−6.8 ± 7.2	NS	−12.1 ± 6.6	NS
3.1×10^{-6}	−8.3 ± 3.4	NS	−11.4 ± 0.7	NS
3.9×10^{-6}	−9.8 ± 4.3	NS	−11.0 ± 3.8	NS

*Values = mean ± SE of 5 experiments. Data were analyzed by Student's t test for paired samples, comparing both series of data (during and after perfusion) with basal values. NS = not significant.

groups of five animals. Data were analyzed by Student's t test for paired samples, assuming the 0.05 level of probability as significant; the dose-response relationship was obtained by means of linear regression analysis; and the probability of significant regression was computed by analysis of variance.

In vivo studies.—Unanesthetized rabbits were treated by 2-minute injections (ear veins) with different doses of DHAD (1.2, 2.4, and 3.6 mg/kg). ECGs were recorded for at least 1 hour following administration by means of needle electrodes inserted into the limbs. Each dose was tested on different groups of five animals.

RESULTS

In electrically paced isolated rabbit hearts, DHAD caused a significant dose-related decrease of LVP ($y = 13.3 + 7.6x$; $r = 0.98$, $P < 0.02$) and dP/dt_{max} ($y = 32.5 + 4.02x$; $r = 0.99$, $P < 0.01$), whereas no effects were seen on coronary flow. Decrease of both these parameters begins 1-3 minutes from DHAD administration and stabilizes to a plateau within 10 minutes. Interruption of DHAD perfusion during this phase caused a recovery in inotropism within 5 minutes. A second administration of DHAD, after 20 minutes of perfusion without the drug, caused similar but less marked effects than those previously described; however, these effects were seen with the highest doses only (table 1). No effects were seen with a third administration of the drug and with all tested doses.

Administration of DHAD to unanesthetized rabbits, at a dose level even twofold to threefold higher than those employed in clinical practice, did not induce any ECG abnormality in these animals.

DISCUSSION

The present study demonstrates that DHAD, in the range of doses close to human therapeutic concentrations (14), causes, in the isolated and perfused rabbit heart, a dose-related and reversible impairment of cardiac contractility, qualitatively similar to that described with doxorubicin (8). However, unlike this latter drug, the administration of DHAD, even at high doses, did not induce any ECG alterations in the normal rabbit.

The trend of the phenomenon we observed, ie, the lessening of negative inotropic effects during a second administration and the absence of these effects during a third administration of DHAD, strongly suggests that this molecule is able to induce tachyphylaxis, probably due to a release of some mediator(s). Interestingly, the same phenomenon has been described with doxorubicin: in fact, Bristow et al (12,13) and Stewart et al (14) report that the acute cardiac toxicity induced by this drug in dog and rabbit is due to histamine release which, in turn, induces a release of other vasoactive substances. According to these investigators, these effects, which occur after every single administration of doxorubicin, could lead to the onset of chronic cardiomyopathy (15). Moreover, since some data from our laboratory (16) and those of other investigators (2) have shown that the chronic administration of DHAD causes, in both animals and humans, alterations of cardiac morphology qualitatively similar to those of doxorubicin, it is to be supposed that both of these drugs may have a similar spectrum of myocardial activity and that they might share the mechanism(s) by which they induce these cardiac effects.

However, some of our previous studies (11) question the role of histamine as a main biochemical determinant of doxorubicin-induced chronic cardiomyopathy; in fact, our experiments have shown that the administration of doxorubicin, at a dose level able to induce cardiac damages in the mouse, did not cause a massive histamine release and that repeated administrations of high doses of histamine did not induce any alteration of cardiac morphology similar to that caused by doxorubicin.

Finally, it has to be stressed that another proposed mechanism by which doxorubicin may cause chronic effects, ie, free-radical production (17), cannot account for the DHAD-induced cardiomyopathy since it has been demonstrated that DHAD, unlike doxorubicin, inhibits free-radical production (18) and/or the hydroperoxide-dependent initiation and propagation reactions in fatty acid peroxidation (19).

On the basis of these studies, it would not be anticipated that DHAD could elicit its cardiotoxic effects by the same mechanism(s) involved in the onset of anthracycline-induced cardiomyopathy.

In summary, our data suggest that DHAD has acute cardiotoxicity similar to that of doxorubicin and that

the rabbit is a useful animal model to investigate the cardiac actions and the related pathogenetic mechanism(s) of this new antitumor drug.

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BRIEF REPORTS

High-Dose Etoposide Therapy for Extensive Small Cell Lung Cancer: A Cancer and Leukemia Group B Study[1,2,3]

Sharon D. Luikart,[4,*] Kathleen J. Propert,[5] Caron R. Modeas,[6] Mark R. Green,[7] and Michael C. Perry[8]

Twenty-one previously untreated patients with extensive small cell lung cancer were treated with etoposide, 400 mg/m^2/day for 3 consecutive days. Myelosuppression was severe, with a treatment-related death rate of 28%. Five partial responses were achieved. High-dose etoposide as given in this study produced unacceptable toxicity and no complete responses. [Cancer Treat Rep 71:533–534, 1987]

Survival of patients with extensive small cell lung cancer (SCLC) has not increased in recent years, and the percentage of long-term survivors remains < 10% (1,2). New chemotherapy regimens and schedules are necessary to improve complete response rates and response duration. Etoposide is one of the more active single agents for the treatment of SCLC (2), and in vitro experiments using L1210 tumor cells have suggested a dose-response relationship (3). Clinical studies have also suggested that frequently repeated high-dose etoposide has improved response rates in SCLC (4). In a pilot study at Vanderbilt University of ten evaluable patients treated with etoposide at a dose of 400 mg/m^2/day iv for 3 consecutive days, the objective response rate after two cycles was 80%, including 40% complete responses (5,6). This was superior to combination regimens (2) and to standard-dose etoposide, which has an objective response rate of 40%–50% (2,4,7). Thus, Cancer and Leukemia Group B (CALGB) sought to investigate, in a phase II study, the activity of high-dose etoposide in ambulatory, previously untreated patients with extensive SCLC.

MATERIALS AND METHODS

Patients with extensive, histologically or cytologically documented SCLC were eligible for the study. Extensive disease was defined as tumor not confined to a lung, contralateral hilar nodes, or ipsi- or contra-lateral supraclavicular nodes. A malignant pleural effusion was also considered extensive disease. Measurable disease, a performance status of 2 or better (CALGB criteria), a life expectancy of at least 2 months, and no prior chemotherapy or radiotherapy were also required.

Twenty-one patients (15 males and 6 females) consented to treatment and were entered in the study between May and October 1985. Patient characteristics are described in table 1.

The drug schedule was etoposide, 400 mg/m^2/day iv

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[2] This study (8533) was conducted by Cancer and Leukemia Group B (*Emil Frei, III*, chairman, CA-31946) and was supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, and by a grant from the T. J. Martell Foundation for Leukemia and Cancer Research. The following institutions participated in the study: Bowman Gray School of Medicine, Winston-Salem, NC (CA-03927); Central Massachusetts Oncology Group, Worcester, MA (CA-37135); Columbia University College of Physicians and Surgeons, New York, NY (CA-12011); Dana-Farber Cancer Institute, Boston, MA (CA-32291); Dartmouth-Hitchcock Medical Center, Hanover, NH (CA-04326); Massachusetts General Hospital, Boston (CA-12449); University of Minnesota Medical School, Minneapolis (CA-16450); University of Missouri-Columbia Hospital (CA-12046); Mt. Sinai Hospital, New York, NY (CA-04457); New York Hospital, NY (CA-07986); Rhode Island Hospital, Providence (CA-08025); University of California San Diego Medical Center (CA-11789); University of Maryland Cancer Center, Baltimore (CA-31983); Upstate Medical Center at Syracuse, NY (CA-21060); and Walter Reed Army Medical Center, Washington, DC (CA-26806).

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TABLE 1.—Patient characteristics

Characteristic	No. of patients (%)
Sex (M/F)	15/6
Median age in yrs (range): 62 (50–69)	
Performance status	
0	7 (33)
1	8 (38)
2	6 (29)
Disease sites at entry	
Chest	21 (100)
Distant nodes	3 (14)
Pleura	7 (33)
Bone	10 (48)
Bone marrow	9 (43)
Liver	7 (33)
Brain	5 (24)
No. of metastatic sites/patient	
1	8 (38)
2	7 (33)
3	2 (10)
4	4 (19)

for 3 consecutive days, repeated every 4 weeks. After two cycles, the patients were re-evaluated, and those with objective responses continued on therapy for an additional six cycles. Standard CALGB response criteria were utilized.

RESULTS

All 21 patients entered were evaluable for toxicity and tumor response. One patient who had hemoptysis requiring radiation therapy and one patient with a perforated bowel were removed from study during the first cycle and have been considered nonresponders. Five partial responses (24%) and no complete responses were observed. Thus, the 95% confidence interval for objective response is 6%–42%. Response duration ranged from 30 to 144 days. Four of nine patients who were subsequently treated with other combination chemotherapy regimens then had objective responses. However, there were no responses seen in four patients subsequently treated with cyclophosphamide, doxorubicin, and vincristine. Median survival for all 21 patients was 139 days.

This etoposide schedule produced severe myelosuppression in the patient population. Thirteen patients (62%) had wbc count nadirs $< 1000/mm^3$ with a median nadir of $600/mm^3$, and 16 patients (76%) had platelet count nadirs $< 100,000/mm^3$ with a median nadir of $49,000/mm^3$. Six patients had severe anemia, 12 had life-threatening infections, and two had severe hemorrhages. Sixteen patients required hospitalization for treatment-related toxic effects. Six patients died with myelosuppression and pneumonia, five during the first cycle of etoposide and one partial responder during the third cycle. Thus, treatment-related deaths occurred in 28% of the patients. Additionally, one patient had a transient elevation of SGOT noted on the third day of therapy, one had severe mucositis, and four experienced total alopecia.

DISCUSSION

In this group of 21 previously untreated ambulatory patients with extensive SCLC, high-dose etoposide produced severe bone marrow toxicity and an unacceptably high rate of treatment-related deaths. Therefore, we terminated the study prior to attaining our projected accrual of 45 patients. Wolff (5) previously reported 13 patients treated similarly, with three deaths during the first cycle of treatment, all in nonambulatory patients; all were neutropenic and febrile at the time of death. Our stricter entry criteria did not prevent treatment-related fatalities. The response data are also disappointing, since there were no complete responders in the surviving patients. Thus, we cannot recommend etoposide in this dose and schedule for the treatment of patients with extensive SCLC.

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Phase II Study of Idarubicin in Advanced Endometrial Carcinoma[1,2]

Thomas B. Hakes* and Virginia Raymond[3]

Idarubacin (DMDR), an anthracycline analog (1), was selected for clinical trial because anthracyclines are active in endometrial cancer (2), DMDR is more potent and less cardiotoxic than daunorubicin in animal models (3,4), and DMDR may be administered both iv and orally (3). Phase I trials established myelosuppression as dose-limiting for DMDR and determined 12.5 mg/m^2 iv every 3 weeks as an initial dose for phase II trials (5).

All patients had advanced adenocarcinoma of the endometrium with bidimensionally measurable disease, Karnofsky performance status $\geq$ 50%, no clinical or laboratory evidence of cardiac dysfunction, serum bilirubin $<$ 1.5 mg/dl, and serum creatinine $<$ 2.5 mg/dl.

Among the 25 patients studied (table 1), 23 had had prior radiation therapy and nine had had prior chemotherapy (two with doxorubicin combinations and seven with melphalan and 5-fluorouracil).

In patients with normal marrow reserve, DMDR (12.5 mg/m^2) was given as an iv bolus every 3 weeks. In marrow-impaired patients, the dose was reduced to 10 mg/m^2 and escalated to 12.5 mg/m^2 when no myelosuppression occurred.

Twenty-four of 25 patients treated were evaluable for toxicity and 21 of 25 were evaluable for therapeutic response. One patient was lost to follow-up after the first dose and was inevaluable. The median number of DMDR doses was three (range, one to 13).

Two of 21 (10%) (95% confidence limits, 2%–30%) patients evaluable for response achieved a partial remission lasting 6 and 9 months. Two patients had minor responses lasting 4 and 6 months. Three patients had stable disease and 14 had progressive disease. The response rate was 14% among seven patients with prior chemotherapy and 7% among 14 patients with no prior therapy. Because of the difficulty of evaluating recurrent pelvic disease after radiation therapy, it is of note that only five patients had pelvic disease as the sole site of measurable disease.

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total	25
Evaluable for response	21
Evaluable for toxicity	24
Response	
Partial	2
Minor	2
Stable disease	3
Disease progression	14
Median age in yrs (range): 62 (36–71)	
Median Karnofsky performance status (range): 70% (50%–90%)	
Prior therapy	
None	1
Chemotherapy	1
Chemotherapy + radiation therapy	8
Radiation therapy	15
Sites of metastatic disease	
Lung	10
Pelvic, vagina	9
Liver	5
Abdominal	4
Superficial lymph node	2
Skin	1
Subcutaneous	1
Pelvic and/or vagina as sole site of measurable disease	5
Toxicity	
Myelosuppression (cells/mm^3)	
Median wbc count nadir (range): 2800 (400–16,100)	
Median platelet count nadir (range): 246,000 (23,000–618,000)	
Nausea	18
Vomiting	6
Anorexia	8
Alopecia	12
Marked alopecia	1
Fatigue	4
Diarrhea	2

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Toxicity was minimal. Myelosuppression was the most frequent side effect, with a median leukocyte count of 2800/mm^3 appearing at a median of 15 days. Nonhematologic side effects were mild, either grade 1 or 2 (World Health Organization scale). No clinical cardiotoxicity was seen. Three patients who received long-term therapy had normal cardiac radionuclide cineangiography studies following cumulative doses of 70, 82, and 150 mg/m^2.

Doxorubicin has recognized antineoplastic activity in endometrial cancer. Response rates of 19% and 37% have been reported (2). DMDR has demonstrated activity in previously treated patients with leukemia (3). The drug is particularly attractive because of its reduced cardiotoxicity in animal systems and its potential for oral administration. However, in the group of minimally to moderately pretreated endometrial cancer patients presented here, only modest antitumor activity was observed. We therefore conclude that at the route, dose, and schedule used DMDR has modest activity in endometrial carcinoma.

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Androgen Therapy in the Treatment of Refractory Epithelial Ovarian Cancer[1]

John J. Kavanagh,[2,*] J. Taylor Wharton,[3] and William S. Roberts[2]

Androgens are steroidal derivatives of testosterone. Antitumor responses with these compounds have been noted in patients with breast carcinoma and occasionally in hypernephroma. Several theories have been proposed to explain this antitumor activity. There may be reduction or inhibition of prolactin receptors. Other possibilities include inhibition of estrogen synthesis, either from adrenal or peripheral precursors. There may also be competitive inhibition of estrogen receptor sites (1). Epithelial ovarian carcinoma frequently demonstrates the presence of estrogen and progesterone receptors (2). Clinical trials of tamoxifen and progestational agents have yielded modest response rates (3). Androgen binding has also been detected in a large percentage of epithelial ovarian carcinomas (4,5). For these reasons, an empirical trial of androgen therapy in patients with refractory epithelial ovarian cancer was undertaken.

MATERIALS AND METHODS

Sixteen patients who had failed prior surgical debulking attempts and combination chemotherapy received therapy. Only patients with epithelial ovarian cancer were eligible. The androgen used was fluoxymesterone at a dose of 10 mg orally twice a day. Six weeks of therapy was considered a minimum acceptable treatment duration. Hormonal receptor studies were not done.

All patients had received cisplatin combinations. Four patients had received estrogen replacement therapy at least 1 year prior to beginning the androgen. Five patients had a previous therapeutic trial of tamoxifen, progestins, or estrogen combined with progestins. The metastatic sites and histologic grade of the tumor were usual for this patient population. Patient characteristics are shown in table 1.

TABLE 1.—Patient characteristics

Characteristic	No. of patients (%)
Evaluable	16 (100)
Median age in yrs (range): 55 (35–80)	
Performance status (Zubrod)	
0	1 (6)
1	6 (38)
2	7 (44)
3	2 (13)
Prior chemotherapy	16 (100)
Cisplatin	16 (100)
Median No. of regimens: 1	
Prior hormonal agents	
Estrogen replacement	4 (25)
Therapeutic	5 (31)
Metastatic sites	
Pelvis/abdomen	15 (94)
Ascites	3 (19)
Lung/pleura	0 (0)
Lymph node	1 (6)
Histologic grade	
I	2 (13)
II	6 (38)
III	5 (31)
Unknown	3 (19)
Histologic type	
Serous	6 (38)
Mucinous	1 (6)
Endometrioid	3 (19)
Undifferentiated	1 (6)
Unclassified	5 (31)

RESULTS

Of the 16 patients treated, all are considered evaluable. Patients tolerated the androgen well. Two patients developed increased noticeable facial hair. Two patients developed a round-faced appearance. Two patients developed nausea with the compound. Most patients reported an improvement in appetite and strength. No patient had an objective complete or partial response. Fluoxymesterone administered to patients with refractory epithelial ovarian cancer who have received cisplatin combination chemotherapy is not an effective treatment.

[1] Received Oct 1, 1986; revised Dec 15, 1986; accepted Jan 12, 1987.

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Phase II Trial of Anaxirone in Advanced Non-Small Cell Lung Cancer[1]

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Anaxirone (1,2,4-triglycidylurazol) is a new triepoxyd with alkylating properties which has been shown to be active in a number of experimental murine tumors (1,2). In the clinical phase I evaluation, the dose-limiting toxicity was delayed leukopenia and thrombocytopenia. Furthermore, dose-related gastrointestinal toxicity and dose-unrelated phlebitis of mild to moderate degree have been observed (3). In the phase I trials, partial remissions were observed in two patients with non-small cell cancer of the lung and in two patients with metastases of an adenocarcinoma with unknown primary origin (3). This study was undertaken to determine if objective responses can be achieved with anaxirone in good-risk patients with advanced non-small cell cancer of the lung and to further characterize the side effects of this drug.

Three institutions of the Phase II Study Group, Association of Medical Oncology, German Cancer Society, entered 16 patients with histologically confirmed non-small cell lung cancer into this trial. Nine patients received anaxirone at the dose of 800 mg/m^2, and five patients received 600 mg/m^2 as an iv push injection every 4 weeks. Two patients receiving prior radiotherapy to the primary tumor were treated with 650 mg/m^2. Tumor response and toxicity were assessed according to World Health Organization (WHO) criteria (4).

Patient characteristics, responses, and toxic effects are summarized in tables 1 and 2. No objective complete or partial remission was observed in 14 fully evaluable patients with non-small cell lung cancer. These patients demonstrated favorable prognostic factors responding to chemotherapy, ie, a good performance status (0–2 according to WHO) and no prior chemotherapy. A lack of response in 14 patients indicates that the probability of anaxirone having an activity of at least 20% is $< 5\%$. Four patients showed stable disease after two cycles. Two of these four patients showed progressive disease after the third cycle, whereas the two remaining patients refused further treatment due to anorexia.

TABLE 1.—Patient characteristics and response after 2 cycles of treatment

	No. of patients
Entered	16
Median age in yrs (range): 58 (41–69)	
Male/female	12/4
Carcinoma	
Squamous cell	8
Adenocarcinoma	7
Large cell	1
Disease	
Limited	4
Extended	12
Recurrent primary tumor	10
Sites of metastasis	
Lung	4
Bone	3
Liver	2
Lymph node	2
Pleura	2
Skin	1
Performance status (WHO)	
0	2
1	8
2	6
Mean: 1.25	
Prior radiotherapy	2
Prior chemotherapy	0
Response	
Evaluable	14
Treatment cycle	
1 only	4
2	7
3 and more	5
Complete and partial remission	0
No change	4
Disease progression	10
Early death*	2

*Due to toxicity and tumor progression.

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[2] Phase II Study Group of the Association of Internal Medicine, German Cancer Society.

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TABLE 2.—Toxic effects*

	No. of patients
Evaluable	16
Leukopenia	
0	8
1	3
2	4
3	0
4	1
Thrombocytopenia	
0	13
1	1
2	1
3	1
4	0
Hemoglobin decrease	
0	9
1	5
2	2
Gastrointestinal toxicity	
0	3
1	4
2	9
3	0
Anorexia	
> 4 days	2
Alopecia	
0	14
1	2
2	0
Local thrombophlebitis	3

*WHO grades, after the first cycle.

The major side effects were leukopenia and gastrointestinal toxicity consisting of vomiting and anorexia despite antiemetic treatment. One drug-related death was encountered in a 50-year-old woman who presented no risk factors, especially no indication of bone marrow or pronounced osseous metastases. She died in septic shock due to agranulocytosis (200 leukocytes/mm^3). Because of this drug-related death, the next five patients received the reduced dose of 600 mg/m^2.

The frequency of hematologic side effects, which were dose-limiting in the phase I studies (3), was markedly lower in these good-risk patients in comparison to pretreated women with advanced breast cancer. In the last study, the dose of 600 mg/m^2 iv once every 4 weeks led to severe myelosuppression with discontinuation of therapy in 17 of 23 patients (5). We conclude that anaxirone in the present schedule and dose has no activity in good-risk patients with advanced non-small cell cancer of the lung.

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Phase II Study of *N*-Methylformamide in Patients With Advanced Colorectal Cancer[1,2]

N. Simon Tchekmedyian,* R. S. Kaplan, M. Eisenberger, J. Abrams, and D. Van Echo[3]

Despite extensive testing, the role of chemotherapy for the treatment of advanced colorectal carcinoma remains unsatisfactory, and therefore phase II trials with new agents are indicated. *N*-Methylformamide (NMF) is a polar-planar solvent with cytotoxic and differentiating effects in preclinical studies (1,2). Phase I studies indicate that the dose-limiting toxic effects are hepatotoxicity and constitutional symptoms such as fatigue and decreased performance status (3,4). In this study NMF was given to 14 patients with measurable, advanced colorectal cancer who had not received prior chemotherapy.

Patients were eligible if they had histologically proven colorectal adenocarcinoma, unresectable and measurable lesions, no other malignancy, no prior or concurrent chemotherapy, performance status of 0–2, and life expectancy > 2 months. Required initial laboratory data were wbc count > 3500/μl, platelet count > 100,000/μl, hemoglobin > 10 g/dl, BUN < 25 mg/dl, creatinine < 1.5 mg/dl, SGOT < 180 IU/L, SGPT < 216 IU/L, and bilirubin < 3.0 mg/dl. Patients were followed weekly with history and physical examination, assessment of performance status and clinical toxicity, tumor measurements, cbc with differential, serum BUN, creatinine, SGOT, SGPT, bilirubin, and other tests as clinically indicated. Responses were assessed by computerized tomographic scans of liver metastases (eight patients) or extrahepatic intra-abdominal metastases (two) and by physical examination of measurable malignant hepatomegaly (four).

NMF was administered iv over 30 minutes daily for 5 days and courses were repeated every 4 weeks. Two patients received the initial dose of 1000 mg/m^2/day × 5. Due to hepatotoxicity, the starting dose was subsequently reduced to 800 mg/m^2/day × 5; eight patients received this dose. For patients with moderate liver dysfunction (SGOT, 60–180 IU/L; SGPT, 110–216 IU/L; or bilirubin, 1.5–3.0 mg/dl) prior to treatment, the starting dose was 400 mg/m^2/day × 5. In all patients subsequent doses were reduced by 50% for drug-induced moderate liver dysfunction.

There were no objective responses. All 14 patients evaluable for response had documented progressive disease after one or two courses of therapy. Five of the patients received one course of therapy and nine received two courses (table 1).

Toxicity was substantial (table 1). Moderate to severe nausea and vomiting occurred in most patients. Elevations of SGOT and SGPT occurred in 11 patients. In patients with normal pretreatment SGOT, SGPT, and bilirubin, liver toxicity was mild and reversible. Four patients with elevated pretreatment SGOT and SGPT (two of whom also had an elevated serum bilirubin), however, had severe liver toxicity; all four of these patients had liver metastases and two of them died in liver failure with flapping tremor, encephalopathy, and high bilirubin levels. Elevations of the prothrombin

TABLE 1.—Patient characteristics and results

Characteristic	No. of patients
Entered	14
Median age in yrs (range): 59 (49–79)	
Sex	
Male	11
Female	3
Extent of disease	
Liver metastases	12
Other	2
Median performance status*	
0	5
1	6
2	3
Prior radiation therapy	1
Prior chemotherapy	0
Evaluable for toxicity	14
Evaluable for response	14
NMF dose (mg/m^2)	
1000	2
800	8
400	4

continued

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[2] Supported in part by contract N01-CM-57734 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

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TABLE 1.—Patient characteristics and results (*continued*)

Characteristic	No. of patients
No. of NMF treatments	
1 course	5
2 courses	9
Toxicity	
Gastrointestinal	12
Grade 1 nausea	4
Grade 2 nausea and vomiting	6
Grade 3 nausea and vomiting	2
Hepatic	11
Patients with normal baseline LFTs† ($N = 10$)	7
Grade 1 (1.25–2.5 × normal)	5
Grade 2 (2.6–5.0 × normal)	1
Grade 3 (5.1–10 × normal)	1
Grade 4 (10 × normal)	0
Patients with elevated baseline LFTs ($N = 4$)	4
Grade 1—mild (1.25–2.5 × normal)	0
Grade 2—moderate (2.6–5.0 × normal)	0
Grade 3—severe (5.1–10 × normal)	3
Grade 4—very severe (10 × normal)	1
Decreased performance status by at least 1 ECOG grade	12
Fatigue/malaise	10
Mild	3
Moderate	4
Severe	3
Anorexia	10
Mild	1
Moderate	5
Severe	4
Abdominal pain	7
Mild	2
Moderate	2
Severe	3
Response	
Complete	0
Partial	0
Stable disease	0
Progressive disease	14

*Eastern Cooperative Oncology Group (ECOG) scale.
†LFTs = liver function tests (SGOT and/or SGPT).

time of at least 3 seconds over the normal baseline levels were documented in these two patients. This was in spite of a reduced dose of 400 mg/m^2. This suggests that patients with liver metastases and increased pretreatment serum liver enzyme levels (SGOT; SGPT) should not receive NMF, even at reduced doses. Hepatotoxicity has in fact been the main toxic effect observed in both animal and human studies (3).

Abdominal pain, usually localized to the right upper quadrant at the end of each 5-day course of therapy, developed in seven patients and lasted 2–3 days. In two patients, the pain required narcotic analgesics. Worsening in performance status was observed in 12 patients and fatigue, malaise, weakness, and anorexia were universal findings. No hematologic or renal toxic effects were observed. NMF was inactive in colorectal cancer in a previous study (5), and similarly in our trial, NMF given by 5 daily 30-minute iv infusions did not show activity in 14 previously untreated patients with colorectal carcinoma. These results indicate that NMF is inactive and does not deserve further testing in patients with advanced colorectal cancer.

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Evaluation of Fludarabine Phosphate in Renal Cell Carcinoma: A Southwest Oncology Group Study[1,2]

Lodovico Balducci,[3] Brent Blumenstein,[4] Daniel D. Von Hoff,[5] Marilyn Davis,[3] Henry E. Hynes,[6] Ronald M. Bukowski,[7] and E. David Crawford[3,*]

The activity of fludarabine phosphate was explored in 36 patients with advanced renal cell carcinoma. This 2-fluoro-5'-phosphate derivative of vidarabine appears to inhibit DNA synthesis after phosphorylation to the triphosphate derivative by deoxycytidine kinase (1,2). It was found that fludarabine phosphate at the doses administered had no activity in renal cell carcinoma.

MATERIALS AND METHODS

Patients.—Thirty-six patients with pathological diagnosis of renal cell carcinoma were entered in the study (table 1). Criteria for eligibility were no previous chemotherapy, presence of measurable disease, life expectancy of at least 6 weeks, Southwest Oncology Group (SWOG) performance status ≤ 3, serum bilirubin ≤ 2.0 mg/dl, serum creatinine ≤ 1.5 mg/dl or creatinine clearance $\geq$ 60 ml/minute, and pretreatment wbc count $\geq 3500/\mu l$ or platelet count $\geq 100{,}000/\mu l$. All patients signed a written informed consent prior to entering the study.

Treatment plan.—Fludarabine phosphate was given by rapid iv bolus at 25 mg/m^2/day for 5 days every 28 days. Dose escalations of 25% were performed if nadir platelet counts were $\geq 100{,}000/\mu l$ and granulocyte counts were $\geq 1500/\mu l$. A 25% dose reduction was instituted for nadir platelet counts $\leq 50{,}000/\mu l$ or granulocyte counts $\leq 1000/\mu l$. Treatment was discontinued in the presence of progressive disease or in the absence of response following two full courses.

Evaluation criteria.—Complete remission was defined as the disappearance of all clinical evidence of active tumor for a minimum of 4 weeks; partial remission was defined as a $\geq 50\%$ decrease in the sum of the products of all diameters of measurable lesions, without increasing size of any lesion and in the absence of new lesions, for 4 weeks or longer; stable disease was defined as steady state or minor changes in tumor size ($< 25\%$ increment or decrement), lasting a minimum of 8 weeks; and disease progression was defined as an increase of at least 25% in the size of any measurable lesion, appearance of new lesions, or uncontrolled hypercalcemia.

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total	36
Males/females	28/8
Median age in yrs (range): 60 (24–78)	
Measurable disease	
Lung	26
Kidney	17
Liver	2
Intra-abdominal or intrapelvic masses	5
Superficial lymph nodes	4
Subcutaneous masses	2
Neoplastic lesions	
1	15
2	10
≥ 3	11
Performance status*	
0	7
1	19
2	7
3	3

*SWOG scale.

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TABLE 2.—Toxicity

Toxic effect	No. of patients
Hematopoietic	31
Anemia	22
Granulocytopenia	25
Lymphopenia	29
Thrombocytopenia	3
Gastrointestinal	10
Nausea and vomiting	9
Diarrhea	3
Esophagitis	1
Neurological	2
Somnolence	2

RESULTS

Response.—Six patients were not evaluable: three had early death (2, 2, and 8 weeks in the study), two requested termination of protocol treatment, and one experienced a rise in serum creatinine which prevented further drug administration. No partial or complete responses were observed. Fourteen patients had stable disease by the time the study was terminated.

Toxicity.—Treatment-related toxic effects are shown in table 2. Six patients had life-threatening granulocytopenia (granulocyte count $< 100\ \mu l$) and one had life-threatening thrombocytopenia (platelet count $< 10{,}000/\mu l$). Two treatment-related deaths occurred: one patient died of sepsis during drug-induced neutropenia and the other developed coma and died during drug-induced thrombocytopenia; in the absence of autopsy, his death was attributed to intracranial bleeding.

DISCUSSION

Fludarabine phosphate at the doses used in this study has no recognizable activity in renal cell carcinoma. This drug appears to induce significant hematological toxicity, which may narrow its therapeutic index in malignant diseases. Further evaluation of fludarabine phosphate in renal cell carcinoma does not seem warranted.

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Phase II Trial of Acivicin in Malignant Mesothelioma[1,2]

Geoffrey Falkson,[3,*] Daniel A. Vorobiof,[3] Ian W. Simson,[3] and Ernest C. Borden[4]

Acivicin (AT-125), a fermentation product of *Streptomyces sviceus*, is a glutamine antagonist (1). The antitumor effect of glutamine antagonists is believed to be exerted by inhibition of the glutamine-dependent synthetic enzymes (2). Phase I trials have demonstrated both neurologic and myelosuppressive dose-limiting toxic effects that are schedule-dependent (1).

The median survival time from the onset of symptoms for untreated patients is 18 months (3). No satisfactory treatment has been described for patients with this disease (4,5). The sporadic responses obtained with commercial drugs have not changed the outlook for patients with this disease. Phase II studies of new agents therefore constitute a logical approach to patients with this disease who cannot be cured by surgery.

The present study was undertaken to assess the effectiveness of AT-125 in patients with unresectable malignant mesothelioma.

MATERIALS AND METHODS

Criteria for patient eligibility included histologically confirmed malignant mesothelioma of the pleural or peritoneal cavity, evaluable and/or measurable disease, Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0-2, no prior chemotherapy, and informed consent. Patients were required to have wbc count $\geq 4000/mm^3$, platelet count $\geq 100,000/mm^3$, BUN < 30 mg/100 ml, serum creatinine < 1.6 mg/100 ml, and creatinine clearance ≥ 55 ml/minute. AT-125 was administered at a dose of 20 mg/m^2/day given as a 72-hour iv infusion every 21 days. Dose modification was performed in patients with central nervous system, hematologic, and/or kidney toxic effects.

TABLE 1.—Patient characteristics*

Characteristic	No. of patients
Median age in yrs (range): 62 (29-71)	
ECOG PS	
0	3
1	6
2	14
Sex	
Male	16
Female	7
Site	
Pleura	20
Peritoneum	2
Both	1
Histologic type	
Mixed	5
Sarcomatous	2
Epithelial	5
Unknown	11
Prior radiotherapy	2
Total dose administered in mg/m^2 (range): 204 (57-858)	
Response	
Stable disease	12
Disease progression	9
Unevaluable†	2
Median time in the study in wks (range): 8 (1 to > 47)	

*Median survival for all patients: 28 wks (range, 1-65). Median survival for patients with PS of 0-1: 35 wks (range, 17 to > 77).

†1 patient had early death; 1 refused treatment.

RESULTS

Patient characteristics are shown in tables 1 and 2. Twenty-three patients were entered in the study. Two patients were not evaluable for response: one patient

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[2] This study was conducted by the Eastern Cooperative Oncology Group (*Paul P. Carbone*, chairman, CA-21115) and was supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

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[4] University of Wisconsin Clinical Cancer Center, Madison (CA-21076).

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TABLE 2.—Toxicity: mild and severe*

Toxic effect	ECOG grade— 1	2	3
Leukopenia	3	0	0
Anemia	2	0	0
Neurologic	4	3	3
Nausea and vomiting	9	3	3
Kidney	1	0	0
Diarrhea	2	1	0
Stomatitis	2	1	0

*Values = No. of patients.

died 1 week after treatment was initiated due to chronic congestive heart failure and the other refused further treatment after one cycle was given.

DISCUSSION

This phase II trial in malignant mesothelioma failed to identify antitumor activity for AT-125. The median survival in this group of patients is similar to that previously reported with other treatments.

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Continuous-Infusion Vinblastine for Treatment of Refractory Adenocarcinoma of the Cervix[1]

John J. Kavanagh,[2,*] Larry Copeland,[3] David Gershenson,[3] and William Roberts[2]

Vinblastine is a vinca alkaloid with a broad spectrum of antitumor activity (1). When given as a 5-day continuous infusion, there are sustained blood levels. Such an infusion technique has resulted in clinical responses in metastatic breast cancer (2). Adenocarcinoma of the cervix is an uncommon malignancy. Tamoxifen and combination chemotherapy with 5-fluorouracil, doxorubicin, and cisplatin have yielded modest response rates (3,4). For these reasons a trial of continuous-infusion vinblastine was undertaken.

MATERIALS AND METHODS

Fifteen patients with refractory or recurrent adenocarcinoma of the cervix were treated with continuous vinblastine infusions. All patients had pure glandular adenocarcinoma without squamous elements. Clear or glassy cell subtypes were excluded. The starting dose was 1.2–1.4 mg/m^2 for 5 days via a central venous catheter. Patients were treated as inpatients or outpatients with ambulatory infusion pumps. The cycle was repeated every 21–28 days. Standard response criteria were used. The lower dose was used for patients with a Zubrod status of 3 or a history of severe myelosuppression during chemotherapy. Dosage escalation was allowed in 0.2-mg/m^2 increments and aimed at producing a nadir granulocytopenia count of 1.0×10^3 cells/mm^3. Patient characteristics are presented in table 1.

TABLE 1.—Patient characteristics

Characteristics	No. of patients (%)
Total evaluable	15 (100)
Median age in yrs (range): 55 (29–69)	
Performance status (Zubrod)	
0	2 (13)
1	8 (53)
2	2 (13)
3	3 (20)
Prior radiotherapy	15 (100)
Prior chemotherapy*	13 (87)
Metastatic sites	
Central/pelvic	12 (80)
Lung	3 (20)
Liver	1 (7)
Lymph node	6 (40)
Bone	1 (7)
Vinblastine dose (mg/m^2)	
1.6–1.8	7 (47)
1.4	4 (27)
1.2	3 (20)
1.0	1 (7)
Granulocyte count nadir (cells/mm^3)	
Median No. (range): 1500 (70–8170)	
Courses $\leq$ 1000: 39%	
Courses $\leq$ 500: 29%	

*Median No. of regimens, 1. Prior agents: 5-fluorouracil, doxorubicin, and cisplatin, 9 (69%); cyclophosphamide and cisplatin, 2 (15%); cisplatin, 1 (8%); and melphalan, 1 (8%).

RESULTS

Of the 15 patients treated, all are evaluable. The total number of courses given was 44 with a median of two per patient. Six (40%) patients received one dose increase while two (13%) tolerated two dosage escalations. Three (20%) patients required reductions due to toxicity.

One (7%) patient had a partial remission of 5 months' duration in metastatic lymph node sites. Three patients were hospitalized during therapy with fever and neutropenia. Constipation was usual and manageable. One patient had nausea and vomiting requiring iv fluids. Continuous-infusion vinblastine in refractory glandular adenocarcinoma of the cervix does not appear active.

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Alprazolam for Phobic Nausea and Vomiting Related to Cancer Chemotherapy[1]

Seven patients with a prior history of aversion to cancer chemotherapy experienced significantly less anticipatory nausea and significantly less vomiting during and after chemotherapy when alprazolam versus placebo was added to the treatment protocol. Patients identified the active agent as significantly more useful than placebo.

Many cancer patients develop anticipatory anxiety, nausea, and vomiting as a side effect of treatment. Sensory events associated with prior treatment often become conditioned stimuli for this iatrogenic phobia. Cognitive aspects of the aversive syndrome range from transient feelings of dread and pretreatment insomnia to a syndrome of pervasive anxiety and dysphoria between treatments (1). Clinicians have applied systematic desensitization (2) and hypnotic suggestion (3) to relieve the aversive syndrome; however, this type of intervention can be time-consuming and frequently is unavailable.

Our experience with alprazolam for treatment of panic disorder with conditioned agoraphobia led us to propose use of the triazolobenzodiazepine to ameliorate anticipatory anxiety and nausea in patients undergoing cancer chemotherapy. We report results of a double-blind crossover placebo-controlled pilot study of alprazolam administration in eight patients receiving cancer chemotherapy.

METHOD

Eight subjects, including seven females and one male with a mean age of 38, participated in order of referral by nurses in the Massachusetts General Hospital Cancer Center. All had previous anxiety related to treatment and were scheduled for at least two further cycles of chemotherapy. The target sample was eight for the pretrial funded by the Upjohn Company. All were well motivated and gave informed consent. Two of ten did not complete the study because they were not prescribed further chemotherapy. All but one were ambulatory.

Patients received alprazolam or placebo in a double-blind AB-BA crossover design for each of two chemotherapy cycles. One patient did not receive chemotherapy after the placebo treatment because her blood counts were too low.

Instructions were to begin the study drug at the dinner meal on the evening prior to chemotherapy and to titrate dosage to produce mild drowsiness. Patients receiving the active agent typically took 0.25 mg at dinner, 0.5 mg at bedtime, 0.5 mg on morning awakening, and 1.0 mg at noon before chemotherapy. Following chemotherapy, they continued 0.5 mg four times daily as tolerated for an additional 2-day period. Subjects were encouraged to continue their usual antiemetic regimen.

A research assistant, J. Clarke, met with all subjects and administered the Morrow Nausea and Vomiting Scale, SCL 90, and Sheehan anxiety scales on the day of chemotherapy and again on Day 5 following chemotherapy. Pill counts were made at each visit. Patients reported pill intake in writing. At completion of the study, prior to breaking the code, J. Clark was asked to speculate in which order the eight subjects received drug and placebo.

RESULTS

Patients' tumors included breast (three), Hodgkin's (two), and colon, lung, and lymphoma. Four patients received alprazolam first; four received placebo first. One patient did not receive chemotherapy after the first cycle with placebo. With alprazolam her symptoms improved compared to initial scores. Data were compared with one-tabled dependent t-tests for the seven subjects who completed the protocol.

Comparison of the Morrow Nausea and Vomiting Scale revealed a significant reduction in prechemotherapy nausea for alprazolam ($x = 0.57$, $SE = 0.43$) versus placebo ($x = 1.57$, $SE = 0.48$) ($t = 2.6$, $df = 6$, $P = 0.02$). Vomiting during and after chemotherapy also was reduced significantly with alprazolam ($x = 2.00$, $SE = 0.90$) versus placebo ($x = 3.86$, $SE = 0.55$) ($t = 2.1$, $df = 6$, $P = 0.04$). Patients gave blind ratings on a four-point scale which were significantly higher for alprazolam ($x = 1.43$, $SE = 0.30$) versus placebo ($x = 3.14$, $SE = 0.46$) ($t = 2.7$, $df = 6$, $P = 0.02$). The difference was not significant either for vomiting before chemotherapy for alprazolam ($x = 0$, $SE = 0$) versus placebo ($x = 0.29$, $SE = 0.29$) ($t = 1.0$, $df = 6$, $P = 0.30$) or for nausea during and after chemotherapy for alprazolam ($x = 3.00$, $SE = 1.00$) versus placebo ($x = 4.29$, $SE = 0.57$) ($t = 1.1$, $df = 6$, $P = 0.30$) although the trend favored alprazolam. In two patients, the antitumor drugs were changed with the second cycle. In both cases, alprazolam was given in the first cycle and patients had less nausea and vomiting

despite the greater emetic potential of the first cycle. (Mechlorethamine, vincristine, and procarbazine compared to mechlorethamine, vincristine, and prednisone; cisplatin and vinblastine compared to cisplatin alone.) Toxicity was measured after each treatment on a scale of 0–3. There was no significant difference between alprazolam and placebo. Mild sedation was reported by three patients taking alprazolam compared to none with placebo.

No significant difference was noted in respect to anxiety level as measured with SCL 90 and Sheehan scales just prior to chemotherapy and 5 days later.

The research assistant guessed accurately the order of active drug versus placebo in seven of eight patients.

DISCUSSION

Alprazolam significantly reduced nausea before and vomiting during and after chemotherapy in patients who had developed an aversive syndrome. Patients experienced only mild sedation. The measures of anxiety focused on the week of chemotherapy. We suspect that this time interval and the influence of other anxiety-provoking variables, which could not be controlled, clouded the specific anxiolytic effect.

Recently, oral or sublingual lorazepam has been used for sedation and control of emesis in patients receiving cancer chemotherapy (4–6). In one report, five patients who had been refusing treatment chose to continue a potentially curative regimen after premedication with 3 mg of oral lorazepam (4). Although researchers have suspected that benzodiazepines are especially helpful for conditioned nausea, these studies have not differentiated patients who had prior chemotherapy from new patients. It is also unclear whether efficacy of lorazepam derives from anxiolytic or amnestic properties.

We began this project with the idea of modifying conditioned anxiety in patients receiving cancer chemotherapy and in the absence of data on lorazepam. Lorazepam has a similar half-life, but more amnestic effect. We selected cancer patients with the aversive syndrome and studied alprazolam because it has been helpful for ambulatory psychiatric patients with panic disorder and agoraphobia.

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Meningioma Preceding Breast Cancer

An association between meningioma and breast cancer was described by Schoenberg et al (1) in 1975. Thirty-six cases of meningioma in patients first treated for breast cancer have been described in the literature (1–7). The development of breast cancer in patients previously diagnosed with meningioma, however, has been only rarely reported (1,3,4,6,8).

CASE REPORT

A 51-year-old woman was admitted to the hospital in December 1982 because of episodes of paresthesia in the fingers of her left hand and in the left angle of her mouth. Neurological examination did not disclose other abnormal findings and a brain computerized tomography (CT) scan showed a right heterogeneous temporoparietal mass. Carotid angiogram showed a lesion with meningeal vascular supply. A right parietal craniotomy was performed in January 1983, and a transitional meningioma was removed. In April 1984, a mastectomy was performed for an infiltrating ductal carcinoma of her left breast. All axillary nodes were positive for metastatic disease. In March 1986, the patient was diagnosed with multiple bone and liver metastases, and she died of progressive disease in November 1986. No hormone receptors were assayed on the meningioma or in the primary or metastatic breast cancer.

DISCUSSION

In a retrospective study of 135 patients with multiple primary tumors, in whom at least one of the tumors was

TABLE 1.—Patients with breast cancer appearing after meningioma (eight cases)

Author (year)	Mos between diagnoses of meningioma and breast cancer	Age at diagnosis of breast cancer
Schoenberg et al (1975)	5	69
	53	49
	306	86
Mehta et al (1983)	23	57
Miller (1986)	21	67
Burns et al (1986)	36	60
Orbuch and Hemsi (1986)	14	60
Current case	15	52

within the CNS (1), the association of meningioma and breast cancer was found to be in excess of the expected rate of second primary cancers. Meningioma and breast cancer share some epidemiologic characteristics like age and sex distribution. Furthermore, both tumors contain sex steroid receptors (9,10) and their growth may occur secondary to hormonal changes (3). In other respects, however, the hormonal characteristics of breast cancer and meningioma are different. Meningiomas present large amounts of progesterone receptors in the virtual absence of estrogen receptors (10), and these tumors do not seem to respond to hormonal treatments with tamoxifen or medroxyprogesterone acetate (11).

A total of 43 cases of the association of meningioma and breast cancer have been described in the literature (1-8). In 36 of the 43 cases of the association described, breast cancer preceded the meningioma. Only seven cases of breast cancer appearing after meningioma have been previously reported (table 1). It has been stated (2,3) that CNS symptoms and signs in a patient treated in the past for breast cancer cannot always be assumed to represent cerebral metastasis, and that meningioma should be ruled out, especially when a solitary brain lesion exists. Conversely, some authors (3,4) have suggested that patients with proven meningioma should be observed closely for subsequent development of breast cancer. These recommendations may have important prognostic and therapeutic implications, as patients with breast cancer and CNS symptoms could accordingly be cured of a meningioma, and patients with meningioma could benefit from early diagnosis of breast malignancies. With the limited data available, however, a predisposition of patients with meningioma to develop breast cancer is not clear. The frequency of breast cancer complicating meningioma could be underestimated. It could be argued that patients with breast cancer are more closely followed for the occurrence of relapse and, at the same time, for second primary malignancies than patients with meningioma, and that this may account for the differences in observation of the two described associations. In the eight cases reported so far, breast cancer developed after a relatively short time (median, 22 months) after the excision of the meningioma. In seven of the eight cases the interval between diagnosis of meningioma and diagnosis of breast cancer was < 5 years, and in five of the eight cases it was < 2 years. Although the short interval between diagnosis of meningioma and subsequent breast cancer in reported cases may suggest a biological association, such an association cannot be proven on the basis of case reports alone, since patients who develop breast cancer 10-20 years after meningioma may never be reported as, by contrast, patients with meningioma after breast cancer are. Thus, the association of breast cancer after menigioma may simply be a matter of case selection.

Only cancer registry-based studies can clarify the issue of the association of meningioma and breast cancer. While there probably exists an excess occurrence of meningiomas in patients treated for breast cancer (1,6), the converse remains to be proved. In view of the published evidence, close follow-up of patients with meningioma for subsequent development of breast cancer should not be done. Analysis of cancer registries focusing on this association should be encouraged.

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High-Dose Busulfan and Amsacrine With Autologous Bone Marrow Rescue in Patients With Acute Myeloid Leukemia in First Remission

High-dose busulfan (16 mg/kg) combined with cyclophosphamide (200 mg/kg) is being used as a preparative regimen for allogeneic and autologous bone marrow transplantation in patients with acute leukemia in complete remission (1). Both busulfan and cyclophosphamide are alkylating agents. It could be worthwhile to combine busulfan with a nonalkylating drug with proven efficacy in the treatment of acute myeloid leukemia (AML). A likely candidate is amsacrine (*m*-AMSA), the third most efficient drug in inducing complete remissions after cytarabine and anthracycline derivatives (2). The nonhematological dose-limiting side-effects of *m*-AMSA are enteritis and stomatitis (2), being uncommon or mild at doses < 600 mg/m^2/course (2). We decided to study the effects of high-dose busulfan and *m*-AMSA as a preparative regimen in autologous bone marrow transplantation in patients with AML in first remission.

Five patients, four males and one female, with AML underwent bone marrow transplantation between September 1984 and December 1985 (table 1). Remission was induced with one or two courses of cytarabine and daunorubicin. Before bone marrow harvesting, the first three patients (Nos. 1, 2, and 3) received one consolidation course; the other two (Nos. 4 and 5) received two courses with the same drugs. Patients 1, 2, and 3 were transplanted 3 weeks after bone marrow harvesting. Patients 4 and 5 received another consolidation course before transplantation 8 weeks after harvesting.

Bone marrow cells were collected with multiple bone marrow aspirations under general anesthesia. The cells with a density < 1.079 were separated using Ficoll-Isopaque density-gradient centrifugation in an IBM cell washer (type 2991-2). The separated cells were suspended in DMSO-containing medium, stored, and thawed prior to reinfusion according to standard methods.

The patients were hospitalized in a single laminar air flow room with reverse isolation. They received parenteral hyperalimentation, irradiated blood component support (30 Gy), oral antibiotics for partial decontamination of the digestive tract (colistin, co-trimoxazole, and amphotericin B), and systemic antibiotic therapy as required.

From Day −9 to Day −6 (with Day 0 as the day of marrow infusion) the patients received busulfan (4 mg/kg/day orally) in four divided doses for a total of 16 mg/kg. On Days −5, −4, and −3, *m*-AMSA was infused in 60 minutes. Patients 1, 2, and 3 received 200 mg/m^2/day (total, 600 mg/m^2). Patients 4 and 5 received 100 mg/m^2/day (total, 300 mg/m^2).

The busulfan and *m*-AMSA regimen was accompanied by mild to moderate nausea and vomiting in all patients, readily manageable with conventional antiemetic therapy. An equivalent of $92\text{-}136 \times 10^8$ (mean, 119×10^8) nucleated bone marrow cells were reinfused without side effects.

Patients 1, 2, and 3 received the higher *m*-AMSA dose. Patient 1 died 5 days after reinfusion because of rapidly progressive septic shock. Subsequently, blood cultures grew *Streptococcus pneumoniae*. Autopsy did not show any abnormality except for signs of sepsis and shock. Patients 2 and 3 developed very severe stomatitis, diarrhea, and paralytic ileus. Patient 2 recovered after 2 weeks of paralytic ileus and complete desquamation of the skin. He relapsed 161 days after transplantation. Patient 3 died in shock 9 days posttransplant. Autopsy did not reveal infection.

Patients 4 and 5 received the lower *m*-AMSA dose. Patient 4 had moderate stomatitis and a 5-day period of paralytic ileus. He recovered after complete desquamation of the skin, but had an early relapse on Day 82. Patient 5 developed very severe mucositis necessitating intubation. He suffered a cardiac arrest, was resuscitated, but developed shock with signs of intracerebral hemorrhage and died 18 days after transplantation. Autopsy showed extensive desquamation in the gastrointestinal tract and lungs and of the skin, bleeding in the gall ducts, and no sign of infection.

The busulfan and cyclophosphamide regimen regularly results in profound pancytopenia of 2-8 weeks' duration. Nonhematologic toxic effects include mild to moderate nausea, vomiting, and diarrhea, severe stomatitis, and mild to moderate partial desquamation of the

TABLE 1.—Clinical data*

Patient No.	Age (yrs)	Granulocyte count† $> 0.5 \times 10^9$/L	Platelet count† $> 50 \times 10^9$/L	Death†	Cause of death
1	46	nr	nr	5	Pneumococcal sepsis
2	46	17	50	516	Relapse (after 161 days)
3	51	nr	nr	9	Shock
4	49	26	nr	84	Relapse (after 82 days)
5	56	nr	nr	19	Cerebral hemorrhage and shock

*nr = not reached until death.
†Days after bone marrow reinfusion.

skin (1). The replacement of cyclophosphamide by *m*-AMSA dramatically increased the severity of the toxic effects in the gastrointestinal tract and skin. In all four patients surviving long enough, very severe stomatitis and diarrhea occurred with fever, paralytic ileus, and shock. In Patient 3, death was directly attributable to this toxicity. In Patient 5, the stomatitis necessitated intubation because of obvious danger of suffocation. The two patients who survived for some months (Patients 2 and 4) experienced complete desquamation of the skin. The beginning of desquamation was also observed in Patient 3, 2 days before his death. Willemze et al (3) reported a similar increase of *m*-AMSA-induced enteral toxicity as we had observed in patients treated with *m*-AMSA combined with high doses of cytarabine. Skin desquamation was not reported (3). Bone marrow recovery after *m*-AMSA and busulfan is not evaluable in our series. The number of patients is too small to comment on efficacy. It does seem to us, however, that the combination of busulfan (16 mg/kg) with *m*-AMSA (600 or 300 mg/m^2) is too toxic to have a place in autografting.

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ANNOUNCEMENTS

Society for Oral Oncology

The recently formed Society for Oral Oncology is accepting applications for membership from clinicians and laboratory investigators active in oncology as it involves the oral cavity/head and neck. Licensed health professionals, including physicians, dentists, dental hygienists, and nurses, are encouraged to participate.

For further information, contact: Carol S. Beckert, DDS, Membership Chairman, Society for Oral Oncology, Affiliated Pediatric Dental Specialists, 3901 Beaubien Blvd, Detroit, MI 48201.

First International Symposium on Organ Directed Toxicities of Anticancer Drugs

Burlington, Vermont, June 4–6, 1987

For further information, contact: Miles P. Hacker, PhD, Vermont Regional Cancer Center, 1 South Prospect St, Burlington, VT 05401 (telephone: 802-656-4414).

Third International Conference on Malignant Lymphoma: Current Status and Prospects

Lugano, Switzerland, June 10–13, 1987

For further information, contact: F. Cavalli, MD, Division of Oncology, Ospedale San Giovanni, 6500 Bellinzona, Switzerland (telephone: 091-23-62-10).

International Society of Paediatric Oncology—SIOP XIX Meeting

Jerusalem, Israel, June 14–19, 1987

For further information, contact: Prof. Rina Zaizov, Chairperson, PO Box 983, Jerusalem 91009, Israel.

8th International Symposium on Carotenoids

Boston, Massachusetts, July 27–31, 1987

For further information, contact: Dr. Richard F. Taylor, 8th International Symposium on Carotenoids, c/o Applied Biotechnology, Arthur D. Little, Inc, Acorn Park, Cambridge, MA 02140.

Seventh Annual Epidemiology Summer Program

Tufts University, Medford, Massachusetts, July 26–August 14, 1987

For further information, contact: The New England Epidemiology Institute, Department SC-4, PO Box 57, Chestnut Hill, MA 02167 (telephone: 617-734-9100).

International Gynaecological Oncology Meeting

Charing Cross Hospital Medical School, London, England, September 2–3, 1987

For further information, contact: Dr. Newlands, Charing Cross Hospital, Fulham Palace Rd, London W6 8RF, England (telephone: 01-748-2040).

Satellite Symposium on Medical Management of Prostate Cancer

Nice, France, September 2–4, 1987

For further information, contact: Dr. M. Namer, Centre Antoine-Lacassagne, 36 Voie Romaine, 06054 Nice Cédex, France (telephone: 93-81-71-33).

First International Paediatric Oncology Nursing Meeting

Jerusalem, Israel, September 13–18, 1987

For further information, contact: Prof. Rina Zaizov, Chairperson, PO Box 983, Jerusalem 91009, Israel.

Second International IST Symposium: Multimodality Treatment of Ovarian Carcinoma

Genoa, Italy, September 24–26, 1987

For further information, contact: P. F. Conte, MD, Scientific Secretariat, Istituto Nazionale per la Ricerca sul Cancro, V. le Benedetto XV-10, 16132 Genova, Italy (telephone: 010-352828/352803).

Hodgkin's Lymphoma—Recent Advances in Basic and Clinical Research International Symposium

Cologne, Federal Republic of Germany, October 2–3, 1987

For further information, contact: Prof. Dr. V. Diehl, Studiensekretariat Morbus Hodgkin, Medizinische Universitätsklinik, Joseph Stelzmann Str. 9, D-5000 Cologne 41, Federal Republic of Germany.

Principles of Epidemiology

Washington, DC, October 28–30, 1987

For further information, contact: The New England Epidemiology Institute, Department SC-4, PO Box 57, Chestnut Hill, MA 02167 (telephone: 617-734-9100).

2nd International Congress on Neo-Adjuvant Chemotherapy

Palais des Congrès, Paris, France, February 19–21, 1988

For further information, contact: S. O. M. P. S., Hôpital de la Salpétrière, 47 Boulevard de l'Hôpital, 75651 Paris Cédex 13, France.

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